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ACUTE EXPERIMENTAL INFECTIONS IN MICE

QUANTITATIVE MICROBIAL STUDIES

BY

TIMO KOSUNEN

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HELSINKI, FINLAND

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Helsinki, March 1959

T. K.

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INTRODUCTION

The fate of microbial parasites in the host organism has always been an object of great interest. It became the subject of numerous studies soon after the discovery of microbes. The earliest observations, based on investigations from pre-bacteriological times, dealt with the distribution of various inert particles, mostly dyes, injected intravenously into animals.

The elimination of both living and dead foreign particles from the circulation seems to occur either by primary phagocytosis of single particles or by secondary phagocytosis of aggregates in capillaries, or by humoral lytic procedures. The activity of the reticulo-endothelial system with the sessile and wandering cells has been studied on many occasions and particularly the role of the liver, spleen, bone-marrow and polymorphonuclear cells has been found important.

The individual properties of both the host organisms and the microbes as well as the dose and route of infection make the process very complex. Most of the early studies were performed with rabbits, some with mice. The best known is perhaps the experiment of Ørskov, Jensen and Kobayashi (70). They infected mice intravenously with *Salmonella typhimurium* bacilli and noted that after the rapid removal of bacilli from the blood the majority of the invaders was localised in the liver and spleen.

For some ten to twenty years the subject was perhaps not so intensively studied. Then the problem of the host-parasite interaction came to the fore again with the rapid development of chemotherapeutic and antibiotic drugs. New technical improvements permitted the re-study of the old problems. At The Rockefeller Institute Dubos and his coworkers and at Cornell

University McDermott and his colleagues have elaborated and standardised a new quantitative bacterial enumeration method and adapted it for many purposes. Initially they investigated typically chronic infections such as tuberculosis.Subsequently studies have been published concerning the quantitative evaluation of acute bacterial infections, and the applicability of these methods to immunological and therapeutical problems.

SURVEY OF THE LITERATURE

QUANTITATIVE EVALUATION OF MICROBES AND INFECTIONS

The counting of bacteria has always rested on a somewhat uncertain basis. The total particle count has been found to differ considerably from the viable count, the latter being the most commonly utilised in investigations. The occurrence of microbes in clusters is known to cause errors the size of which it is impossible to determine with the methods and facilities of today. The dropping of diluted bacterial suspensions on solid media and mixing of microbes in solidifying substrates have been the universal methods employed in counting viable bacteria. Counting bacteria on the surface of solid media gives better and more accurate results the greater the number of replicate cultures used. In order to reduce the distribution to a useful level at least three replicates of each dilution must be inoculated (128). Even such a crude method as the measuring of 0.1 ml inocula on plates with a 1 ml pipette has given results of adequate accuracy and very slight effect on the total error (109). In the evaluation of the errors of the plate counting method Jennison and Wadsworth (48) emphasised the importance of the error that arises in making dilutions of the bacterial suspensions. They also gave a formula for the calculation of the dilution error when using different dilutions.

The investigation of Fenner, Martin and Pierce (25) concerned the enumeration of viable tubercle bacilli both in cultures and in infected tissues. Their aim was to standardise a simple and accurate method to replace the measuring of inoculation doses in mg of microorganisms. They inoculated the plates with 0.1 ml and 0.02 ml drops of bacterial suspensions. This method gave a relatively high degree of accuracy and was applicable even when suspensions of animal organs and their dilutions were

used. Later it was shown that the number of stainable organisms in tubercle bacilli cultures did not differ significantly from the viable count obtained by culturing (24) and good agreement was found also between the viable and stainable counts from the lungs of mice infected with tubercle bacilli provided the staining was done with extraordinary care (2).

The enumeration of microbes from tissues demands scrupulous homogenisation of the organs, and researchers were obviously handicapped until the introduction of rapid and practical homogenisators in medical microbiology.

Large experimental series were not easy to handle with the slow and inconvenient methods. The number of animals which could be thoroughly investigated in a day remained low, and this tended to hamper the attaining of significant results. The survival of animals in experiments and the severity of the lesions are criteria used extensively in bacteriology. In the research of experimental tuberculosis and salmonellosis there has been a trend towards more accurate techniques.

In this survey only a part of the early literature will be discussed and attention will be concentrated on more recent investigations. In 1928 Ørskov, Jensen and Kobayashi (70) indicated that mice could be used for the quantitative evaluation of *Salmonella typhimurium* infection. The authors gave the inoculation dose by the intravenous route. The majority of the bacteria recovered was located in the liver and spleen.

Freund (27) in 1932 enumerated tubercle bacilli in quinea pigs after experimental inoculation of the disease. He recorded lower numbers of bacilli in the spleen and some lymph nodes of the vaccinated animals than in normal ones.

Nine years later Wessels (127) studied the fate of tubercle bacilli in the tissues of rat by enumerating the viable bacteria recovered from different organs. Raleigh and Youmans (77) noted an enlargement and increase in weight of the lungs of mice during experimental tuberculosis. From this basis Ceriotti (10) later developed a method for the quantitative evaluation of the effect of the antituberculous drugs *in vivo*. McKee, Rake, Donovick and Jambor (59) used mice in testing the antituberculous activity of different compounds. According to their standardised procedure, mice were injected intravenously with

tubercle bacilli and the survival time in both groups was recorded. The method permitted an objective evaluation of the results compared with earlier methods based on assessment of the severity of the lesions. Youmans and Youmans (133) showed a linearity between the logarithms of the bacilli dose (in mg) injected and the mean survival time in days. Five years later they were able to record the results from 180 groups; 98 per cent showed survival times of from 10 to 16 days without significant periodical differences in the yearly and monthly means (134).

Pierce, Dubos and Schaefer (73) in 1953 were the first authors properly to investigate the number of viable tubercle bacilli in the tissues of mice inoculated by different routes. Intracerebral and intravenous injections of bacterial suspensions were followed by infections which were very similar at different times, whereas inoculations performed subcutaneously and intraperitoneally caused infections which differed in intensity at various times, and which could not be regulated accurately by the amount of inoculum. The intravenous inoculation gave the most reproducible results and the maximal numbers of viable bacilli in the spleen and lungs of mice were in linear relationship to the virulence of the strains, a factor which had previously been estimated according to the size of the pathological lesions and the survival of the experimental animals.

Following on from this and aware of the need for experimental methods to evaluate the effect of anti-tuberculous treatment, Mc Cune, Tompsett and Mc Dermott (56, 57) in 1956 in two investigations summarising their work showed the usefulness of bacterial enumeration in tissues as a sensitive index of changes during therapeutic procedures. These authors placed the logarithms of microbial numbers graphically as a function of time after the infection, and connected the daily means by smoothed "trend lines" to give a clear picture of the changes occurring in the microbial populations during the infection.

Sever and Youmans (101, 102) made a critical examination of the enumeration of viable bacilli from mice. They concluded that the variation between individual mice was the greatest source of error in determining the microbial content of the organs. For statistical analysis of the results they found

it necessary to investigate no less than five mice for each sampling. The authors emphasised the importance of determining the plating and dilution error in checking the technique; if these errors were small, they accepted that the technical errors were "included in the variation encountered between mice, since the latter is based on the plating procedure".

The quantitative bacilli enumeration technique involved in studies of experimental tuberculosis in mice has been used in several investigations (14, 118, 58, 17, 49, 124, 125, 18, 5, 6, 80, 132). Kanai *et al.* (50, 51, 52, 130, 131) applied a similar method for the evaluation of experimental tuberculosis in guinea pigs and rats.

Berry (8) performed enumerations of *Salmonella typhimurium* bacilli in mice carcasses ground in Waring blendors. Meynell and Meynell (65) used the same kind of organisms and made the enumeration from pooled organs (heart, lungs, spleen, liver, kidneys and peritoneal washings). Using graded inoculation doses, the authors showed that the greater the challenge dose the greater the number of bacilli recovered from the organ pool, and the steeper the rise of the microbial growth curve. A sublethal *Salmonella paratyphi B* challenge dose caused an infection for which a growth curve could be drawn during an observation time of two weeks. In shape the curve resembled staphylococci curves for mice kidneys.

Schaedler and Dubos (90) also enumerated *Klebsiella pneumoniae* organisms in mice, but only for thirty hours after the challenge. In this time, however, they were able to show the infection-promoting effect of previous fasting, which was evident both in the increased mortality and in the higher numbers of bacteria recovered from the organs.

The quantitative studies of experimental infections in mice caused by *Staphylococcus aureus* and *Candida albicans* are surveyed in the following chapters.

EXPERIMENTAL STAPHYLOCOCCAL INFECTION

A rich variety of infection experiments is possible with staphylococci. Most experimental animals can be succesfully infected

with them. In rabbits, 10 to 15 minutes after intravenous injection of the microorganisms, the initially rapid removal of bacteria from the circulation ceases and the remaining weak bacteraemia continues for many hours (81, 82). The staphylococci still recovered from the blood were mostly located in polymorphonuclear leucocytes. Possibly staphylococci are thus protected from removal by the reticulo-endothelial system. Furthermore, there was a decrease in polymorphonuclear leucocytes during the rapid removal phase. Leucocytes seemed to collect in vascular beds in the lungs and viscera, after which the white cells gradually reappeared in the circulation.

One hour after injection, staphylococci were primarily localised in the liver and spleen of rabbits (116), the lungs, bone marrow and omentum contained very small amounts, and the kidneys, suprarenal glands, muscles, brain, testis and thyroid gland a few organisms only. The large quantities of phagocytic cells present in the liver and spleen together with the sinusoidal type of blood flow may contribute to this type of localisation. According to Dyke (21), intravenously injected staphylococci later localise in the kidneys of rabbits, where the bacterial emboli lodge in the small vessels and multiply there. In glomerular tufts the bacteria are phagocytised by endothelial cells, and the appearance of bacteria in the urine does not occur in active excretion but after tissue damage only. In rabbits, pyelonephritis can be regularly produced by intravenous injection of coagulase-positive staphylococci (68).

The relatively low virulence of staphylococci intravenously injected into mice was noted by Smith, Hale and Smith (108) in the form of high LD 50 values which were never less than 200×10^6 viable bacteria. The number of viable staphylococci in the blood was higher after intraperitoneal than after intravenous inoculation, and the infection was more serious in the former group (41). This was supported by Dutton's findings (20). It was noted that the virulence of staphylococci increased when they were pretreated with coagulable human or rabbit plasma and then injected intraperitoneally (108).

In intravenously infected mice Gray *et al.* (37) found an increase in neutrophile leucocytes. The animals failed to gain weight normally, and mortality was 15 per cent.

The staphylococcal infection of mice had been studied for several years at The Rockefeller Institute when Smith and Dubos (105) published the first investigation in 1956 reporting on the use of the bacterial enumeration method in acute infections. In the blood, spleen and liver the number of organisms decreased continually with all seven staphylococcal strains studied, and generally no culturable organisms were found two weeks after the inoculation. The number of staphylococci in the lungs remained steady during the observation time. Correspondingly, Gray *et al.* (37) found microscopically in the heart, liver, pancreas, brain and intestinal mucosa decreasing numbers of focal lesions. In the liver, spleen and lungs an increase in phagocytes was observed concurrently with declining microbial population curves obtained by bacterial enumeration.

Smith and Dubos (105) found no striking differences between coagulase-positive and coagulase-negative staphylococci in the initial removal phase in mice after intravenous inoculation. Several weeks after the challenge it was still possible to recover viable bacteria from various organs regardless of the virulence or coagulase production of the strains. However, the different behaviour of the coagulase-positive and coagulase-negative strains could be detected later. In the kidneys, the strongly coagulase-positive strains multiplied extensively, producing massive abscesses. Microscopically Gray *et al.* (37) showed bacterial gatherings round the tubules and the larger blood vessels 24 hours after the inoculation. Later on a gradually development of suppurative foci was observed. Gorrill (34) had already earlier shown the localisation of coagulase-positive staphylococci in the kidneys of mice after intravenous inoculation, and Smith (104) regarded the manifestation of staphylococci in the kidneys as typical, suggesting that the evaluation of bacterial multiplication in the kidneys or some modification of it could be used for typing the staphylococcal strains. Only strongly coagulase-positive strains caused lethal infection in the experiments of Smith and Dubos (105). When weakly coagulase-positive or coagulase-negative staphylococci were inoculated the infection was never fatal, bacterial multiplication in the kidneys was transitory and the number of viable bacteria lower than in infections induced with strongly coagulase-positive strains.

Mouse plasma was not coagulated by any of the six staphylococcal strains tested by Smith, Hale and Smith (108), and they thus failed to establish any correlation between mouse-coagulase production and mouse-virulence.

In studies of the correlation of mouse-virulence with *in vitro* properties, it was found that α-hemolysin-productive staphylococci were most virulent (98). Christie, North and Parkin (12) regarded this as a more significant indicator of virulence than coagulase production.

The same authors (12) used the local swelling of the thigh caused by intramuscular inoculation of staphylococci as a classification criterion and evaluated the symptom quantitatively by measuring the size of the swelling.

For quantitative studies the experimental model must be reproducible and give responses which can be regulated. According to Gorrill (35) the logarithm of the number of staphylococci in a challenge dose is linearly related to the proportion of kidneys involved in the infection with abscess-formation within 14 days of the challenge.

He regarded it possible to find the smallest dose of bacteria needed to infect 100 per cent of the kidneys. Furthermore, he detected a linear relationship between the size of the dose injected and the number of bacteria culturable from the infected kidneys 30 minutes after the challenge.

In Gorrill's experiments the standard error of logarithmic values in the enumerations from the kidneys was thirty per cent of the mean, which must be regarded as small. In the logarithmic values of his series the 95 per cent confidence limits were within one logarithmic unit.

The enumeration of bacteria seems to provide the possibility also of studying factors of non-lethal effect in a short term experiment. Especially in metabolic and dietary disturbances the application of the numerical evaluation of microbial invaders has given valuable results.

Fasting and a low protein diet in mice lowered the resistance of the host, and increased numbers of staphylococci could be recovered (90). The state was not corrected when the ordinary tap water used as drinking fluid was replaced by 5 per cent glucose — on the contrary the bacterial populations became

slightly more numerous still in these animals (106). Dinitrophenol and thyreoidin, although they shortened the life of the animals, did not increase the number of bacteria in the kidneys, liver and spleen (107).

Wasz-Höckert *et al.* (121, 122, 123) gave 5—8 per cent ethanol as drinking fluid to infected mice. They reported that it shortened the survival time and that bacteria could be recovered from the liver in ethanol-drinking mice longer than in the control animals.

Dubos and Schaedler (15, 16, 91) reported smaller numbers of staphylococci in the kidneys and liver of mice pretreated with heterologous vaccines, endotoxines and bacterial constituents, than in control animals, but the treatment had to be given some days before the inoculation. A certain protection was reflected also in the number of survivors. However, vaccination simultaneously with or after the inoculation heightened the infection, as had been observed earlier in guinea pig tuberculosis by Wasz-Höckert and Backman (119).

In young mice Wasz-Höckert, Oker-Blom and Kosunen (126) observed that the survival time was shortened when zymosan was injected an hour after the challenge with staphylococci, but in adult mice no differences between the treated and control animals were found even with the bacterial enumeration technique.

The quantitative evaluation of staphylococci was the criterion of Prigal and Dubos (76) also in studies of the lowered resistance of the host during allergic shock.

Chlorpromazine hibernation did not alter the bacterial growth curve in the staphylococcal enumeration studies performed by Wasz-Höckert *et al.* (120).

Chabbert *et al.* (11) used staphylococcal infection of mice to study and compare the effect of different antibiotics. With a constant intravenous dose (10^6 bacteria) the infection was very similar in all the mice of a homogenous strain. 24 hours after the inoculation small abscesses were seen in the kidneys and the logarithms of the number of bacteria were approximately 6.2 per g of tissue. Four days from the onset of the infection the number of bacteria in the kidneys showed no great variation from animal to animal, the arithmetic mean being 8.1 per g of

tissue. The appearance of abscesses in the kidneys and survival was used by Martin *et al.* (63) as the criterion of the therapeutic effect of novobiocin.

The applicability of staphylococci enumeration from the organs of experimentally inoculated mice in studies of the effect of antibiotic drugs has been confirmed by several investigators. McCune, Dineen and Batten (55) used novobiocin, penicillin and streptomycin, Simon, McCune, Dineen and Rogers (103) novobiocin, Gourevitch, Hunt, Moses, Zangari, Puglisi and Lein (36) telomycin, erythromycin and penicillin and Hunt and Moses (45) penicillin, erythromycin, streptomycin, tetracycline and kanamycin in the treatment of experimental staphylococcal infection in mice and recorded good therapeutic results in the infectious process in the kidneys, generally the handiest organ to observe. As a rule the authors used almost overwhelming infections that in some cases killed up to 80 per cent of the untreated mice.

Recently, ristocetin has been marketed. It has been reported to be most effective against staphylococci and enterococci, and in mice infected intraperitoneally with staphylococci an increased number of survivors was observed when this drug was administered (38).

EXPERIMENTAL ENTEROCOCCAL INFECTION

In 1921 Dible (13) was concerned with the enterococcal group in a study in which he also summarised the investigations published earlier. The difficulties of making the survey were considerable. The lack of commonly accepted methods at that time often made it impossible to compare the works of different authors.

Some of the strains had been tested for their pathogenicity for mice, usually regarded as questionable. Dible determined the pathogenicity by injecting 1 ml of a 24-hour serum broth culture subcutaneously into white mice. Only six of the thirty three strains tested were pathogenic in some degree. The criterion of these observations was survival, and if the mice died within a few days the strain responsible was classified as pathogenic.

Twenty years later Friedberg (28) found the same difficulties when he studied non-hemolytic streptococci. 13—25 per cent of the enterococcus-like strains were pathogenic to mice after intraperitoneal inoculation (0.2 ml of an 18-hour broth culture).

Gledhill and Rees (32) had noted the spontaneous death of many of their mice when the heating of the animal house was disturbed. They isolated a streptococcus strain belonging to Lancefield's group D but differing in some other respects from typical *Streptococcus faecalis* (did not ferment mannitol, sorbitol, no growth at 45° C, did not tolerate tellurite, did not hydrolyse aesculin). The disease was characterised by hepatic and sometimes intestinal lesions, and the normal liver tissue was replaced by white masses resembling caseation. The disease could be reproduced experimentally by intraperitoneal and intravenous injection of the isolated enterococci, but the inoculation dose had to be rather large, if the intravenous route was used. No lesions were seen in the lungs, kidneys or spleen when the mice died 4—9 days after being injected intraperitoneally with 0.25 ml of an 18-hour broth culture of this enterococcus. The disease was aggravated by cortisone, especially when large, nearly unphysiological doses were given. Two other strains of enterococci isolated from the mice failed to produce the disease described above.

In rats Highman and Altland (42) were able to produce endocarditis in half the animals by intravenous *Streptococcus faecalis* injections. Together with Eagle (43) they performed bacterial enumerations after Waring blendor homogenisation of the heart and kidneys. Using penicillin they showed that the number of bacteria in the organs was smaller the earlier the treatment was begun.

EXPERIMENTAL CANDIDA ALBICANS INFECTION

Particularly generalised moniliasis and moniliasis of the alimentary canal have always been emphasised as alarming reactions, and the possible provoking factors of the increased occurrence of fungal diseases have been sought intensively. Although

Candida albicans frequently belongs to the normal flora of the human body, it possesses a marked potential pathogenicity which becomes very apparent from an examination of experimental infections.

Redaelli (78) in 1924 used several inoculation routes in studying experimental moniliasis in rabbits, guinea pigs, rats and dogs. After intravenous injection, rabbits died on fifth or sixth, guinea pigs and rats on the fourth or fifth post-challenge day, but dogs were resistant to *Candida* infection. Pseudomembranes appeared in the serous cavities and the organisms entered the circulation too. In intravenously injected animals the kidneys, in particular the cortical substance, showed the most prominent lesions. Redaelli believed renal damage to be the cause of death. In the liver the number of foci was low, which he thought to be a consequence of the eliminatory function of the liver and its acid reaction. In some animals macroscopical abscesses were seen in the heart, spleen, adrenal glands, lymphatic mesenteric glands and in the lymphatic glands localised around the big abdominal vessels. In the brain the lesions were always microscopical only. Short and thick filaments were seen in the tissue foci, which often had a central conidial mass. Polymorphonuclear leucocytes were mobilised first in the local reactions, but later big mononuclear cells, epithelioid cells and fibroblasts appeared to prevent the focus from spreading into the surrounding tissues. In the lungs, spleen and liver giant cells also were seen.

The occurrence of kidney lesions in rabbits was confirmed in later investigations (22, 7, 23). Stovall and Pessin (114) suggested that the lesions were produced by "purely mechanical plugging of capillaries and arteries" because *Candida albicans*, the only yeast considered pathogenic, was also the only one that was seen to form pseudomycelia *in vivo*. Using rabbits, also Evans and Winner (23) were able to observe the transformation of yeast cells to filamentous form.

In the search for smaller and cheaper laboratory animals some authors studied the ability of *Candida albicans* to infect embryonated eggs. Large local foci were seen in the membranes when the surface of the chorioallantoic membrane was inoculated with *Candida albicans*. Both filamentous and yeastlike cells were found in the lesions and the embryos died within six

days (66, 67) but showed no mycotic lesions. Intravenous inoculation into the chorioallantoic membrane, on the other hand, was followed by necrotic foci and granulomas both in the membranes and the embryo (69). Non-pathogenic *Saccharomyces*, *Cryptococcus* and *Torulopsis* species failed to grow in the embryonated eggs and Götz and Nasemann (33) saw in this a possibility for studying the virulence and pathogenicity of fungi.

Nowadays, mice are the test animals used most in experimental moniliasis. Before the intravenous inoculation technique was universally known, however, mice were seldom used since the inoculation of small amounts of microbes by the intraperitoneal route failed to produce fatal infections unless the organisms were suspended in mucin (115, 89).

In 1954 Mankowski and Littleton (62) called attention to the mistaken notion that *Candida albicans* was not pathogenic to mice. 24 hours after injection of 0.2 ml of 1:100 diluted yeast cells all the intravenously injected animals were dead, and when a dilution of 1:1600 was used the majority of the animals died on the fourth to sixth day and all by the ninth day. Fuentes, Schwarz and Aboulafia (29) performed enumeration of the cells used in the challenge and showed that an inoculation of 10,000 cells per g/body weight killed all the mice within 15 days.

The manifestations of moniliasis in intravenously infected mice were reported by Adriano and Schwarz (1). They sacrificed mice at intervals and examined the organs and lesions both macroscopically and microscopically. Granulomas, abscesses and interstitial inflammation dominated the pathological picture and the organs showing most alterations were the heart, kidneys, brain and spleen. Renal lesions were regularly seen and, with myocarditis and encephalitis, they were the suggested causes of death. In the liver, lesions were not observed so regularly, and the eyes, salivary glands, adrenal glands, urinary bladder and lungs were normal.

Except for *Candida albicans* the pathogenicity of the *Candida* species is very limited and only seldom have they been found to cause diseases in man. Mankowski (61) compared the pathogenicity for mice of some related yeasts to *Candida*

albicans, using an intravenous challenge dose of 4×10^6 organisms. Most animals infected with *Candida albicans* died within 24 hours, but *Candida stellatoidea* and *Candida tropicalis* were distinctly less pathogenic. *Candida kruzei*, *Candida parakruzei*, *Candida pseudotropicalis* and *Candida quilliermondi* showed no pathogenicity when given intravenously, but some deaths were observed after intracerebral challenge. All *Candida albicans* and some *Candida stellatoidea* strains were the only *Candida* species producing filamentous forms in the subcutaneous tissues of mice, according to Hill and Gebhart (44).

Importance has been attached to the morphological changes of *Candida albicans* in the analysis of the infectious mechanism of moniliasis in mice. Hill and Gebhart (44) observed within one hour of the inoculation "short rudimentary pseudomycelia" and, later, more advanged changes were revealed. Phagocytosis of the yeast-like organisms was a common finding, but few cells in filamentous form were ingested. The authors suggested that "this transformation favors survival of the fungus in the host by a mechanical interference of ingestion by phagocytes". The difficulties of the phagocytosis of filamentous forms were observed also by Young (135).

Endotoxin has been thought to be one of the factors enabling the *Candida albicans* organisms to cause diseases. Some evidence of it was detected in mice by Salvin (88) who injected dead yeast phase cells intraperitoneally with mucin and with tubercle bacilli adjuvant. The lethal effect of the mixture was ascribed to the release of endotoxin from decomposed yeast cells. Confirmatory evidence was demonstrated by Roth and Murphy (86) who used cell-free supersonic extract of *Candida albicans* combined with chlortetracycline.

In the applications of experimental moniliasis in mice, survival has been the most common criterion. This obviates subjective estimation of the results, but the method can be used only in evaluating extraordinarily effective agents and treatments. In addition, the persistence of infections in the kidneys for six weeks, and in some animals up to six months, was noted by Young (135). He supported the opinion of Winter and Foley (129) that the survival of mice was not a reliable guide to the virulence of *Candida albicans* in short term experiments.

A more detailed, but subjective, system for numerical determination of the monilial lesions in mice was presented in 1953 by Scherr (92). The pathological changes in the kidneys and early deaths were given numerical values:

" 0 = no visible pathology
1 = few, isolated lesions
2 = frequent lesions
3 = many lesions
4 = marked dissemination of lesions
5 = very marked dissemination

If the animal died, presumably as result of Candida dissemination, prior to the end of the treatment period, a value 1 was added to the base value 5 for every day prior to the end of the experiment . . . Thus, the dissemination values recorded here are a measure of both severity of infection and mortality rate."

Scherr himself used the system in several investigations concerning hormones (92, 93, 95, 96, 97) and environmental conditions (93, 94).

Important information for experimental workers was the observation of Ansel and Gauthier (3) that male mice were more susceptible than female mice to monilial infection. The effect of sexual hormones has been studied both separately (60) and combined with cortisone (96, 97). Cortisone aggravated the moniliasis in mice (92, 100, 62) and radiation had a similar influence (85).

Several theories have been published to suggesting that the use of antibiotics is an etiological factor in clinical moniliasis. It has been held that one of the principal factors is the decrease in antibiotic-sensitive, normal bacterial flora. The antibiotics and/or the remnants of the dead bacteria have been thought to have some role in promoting the growth and increasing the virulence of the fungi. The destruction of intestinal bacteria, it has been maintained, results in a relative lack of some vitamins normally produced or prepared by these organisms. Antibiotics could in this way have an indirect lowering effect on the resistance of the host. They may in addition have direct deleterious effects on the cells of the host organism. The stimulation of fungal growth by some antibiotics *in vitro* has been demon-

strated, but others are totally ineffective (26, 54, 72, 47). The *in vivo* effects are likewise inconstant, even with one drug (26, 54, 99, 100, 84, 19, 64, 46, 129, 75).

In liquid media intestinal bacteria restricted the growth of *Candida kruzei*, but when the growth of bacteria was suppressed by antibiotics the yeasts increased strongly in growth (72). *Escherichia coli* also has been shown to have an inhibitory effect on the growth of *Candida albicans in vitro.* (83)

In vivo Escherichia coli in sub-lethal amounts protected mice from an intravenously inoculated *Candida albicans* infection which was usually fatal. If the bacilli were given first, they could be injected either intravenously or intraperitonelly, but if the yeast cells were given first only intraperitoneally injected coli bacilli gave the protection (30, 31).

In this connection the question of the role of properdin arises. Rowley (87) reported on the protective effect of cell walls of *Escherichia coli* bacilli against infection by *Escherichia coli* bacilli suspended in mucin given 24 hours later. On the other hand, Pillemer and Ross (74) reported a rapid decrease in the properdin titer of mice after the injection of zymosan, but the transient decrease was followed by a rise in 48 hours to above the normal level.

After the discovery of the pathogenicity of *Candida albicans* for mice, this experimental model has been used in investigations concerning the activity of antifungal agents. Nystatin increased the survival time of mice challenged intravenously with lethal doses of *Candida albicans* (112, 71, 110) and showed protective action against infection by intraperitoneally injected yeast cells suspended in chlortetracycline (9) and oxytetracycline (40).

Lindh and Kiser (53), studying the effect of antifungal agents, noted a decrease of fungi in mice faeces after the *per os* administration of both Candida organisms and the drug. Sternberg *et al.* (112), who examined alterations in the faecal flora caused by nystatin, also studied the kidneys macroscopically and noted lesions in all kidneys of the control mice; 50 per cent of these organs were undamaged in the treated animals.

The antifungal activity of amphotericin B against *Candida albicans in vivo* has been verified in experimentally inoculated

mice (113, 39, 71). Eight gammas per day injected subcutaneously protected nine out of ten mice inoculated intracerebrally, according to Steinberg, Jambor and Suydam (111). The infective organisms continued to be found in the kidneys when cultures from elsewhere were negative (39).

Among the newer antifungal agents, candidin was presented in 1954 by Taber, Vining and Waksman (117). Its antifungal activity *in vivo* was studied by Solotorovsky *et al.* (110) who inoculated mice by the intravenous route with 0.25 ml of a 1 : 15 dilution of 24-hour Candida albicans culture. Fifty per cent of the control mice died within 10 days, whereas 17 out of 24 treated mice lived for 40 days. The mice were treated with 0.0125 — 0.025 mg of candidin per day during the first ten post-challenge days only.

The literature available

— Contained no detailed report of the dependability and reproducibility of results obtained by the enumeration technique in acute infections, although the technique had been used in some studies of experimental staphylococcal infection in mice;

— Showed that very few studies and no microbial enumeration experiments have been made on experimental enterococcal infection in mice;

— Revealed that despite the increased interest in experimental moniliasis in mice no microbial enumeration experiments of this subject have been reported.

PRESENT STUDY

OBJECT

The object of the present study was:

1. To obtain some idea of the reproducibility and reliability of the results obtained by the microbial enumeration technique in acute infections.
2. Using the microbial enumeration technique, to describe the experimental infections of mice caused by different challenge doses of
   — *Staphylococcus aureus*
   — *Streptococcus faecalis* and
   — *Candida albicans.*
3. To apply the microbial enumeration technique in evaluating the effect of some agents which have been suggested as
   — promoting the infection
   — mitigating the infection
   — being of doubtful effect on the infection.

MATERIAL

Mice and their Handling

About 2000 male mice of Swiss Albino Webster strain, imported in 1956, were used in these experiments. At the time of challenge the weight of the mice ranged from 17 to 22 g; the mean weight per group varied from 19 to 21 g.

The mice were housed in 5 and 10 l glass jars. In the smaller jars the number of mice never exceeded ten, in the larger the maximum was twenty. The jars were furnished with a wire netting cover, through which was led the tube from an inverted water bottle.

The principal food consisted of pellets of standard contents (4); some bread, cheese and oat grains were available in addition. Twice a week the animals were given pellets which had been immersed in cod-liver oil for an hour.

The bottom of the glass jars was covered with shavings which were changed daily.

The temperature in the room housing the mice was kept at 19 to 21 °C.

Staphylococcus Aureus

The "Orion" strain used has the phage pattern 29/73 (/7/42E/54). The typing was kindly performed by Dr. Pirjo Mäkelä at this Institute using the basic set of phages recommended in 1958 by the second Meeting of the International Committee on Phage Typing of Staphylococci. The strain is coagulase- (human and mouse plasma) and phosphatase-producing, sensitive to a penicillin concentration of 0.4 units/ml and to a ristocetin concentration of 6.25 γ /ml. It is non-motile, produces orange-yellow pigment, forms acid from glucose, lactose, sucrose and mannitol, causes no hemolysis on blood agar and grows in media containing 10 per cent of sodium chloride.

Streptococcus Faecalis (Enterococcus)

The strain used was isolated from an ulcer on the leg. This strain grows well on tellurite plate (tellurite 1/2500). On blood agar no hemolysis is seen and the strain is non-motile, grows at 10° and 45 °C, resists 30 minutes heating at 60 °C and grows in broth containing 6.5 per cent of sodium chloride. It forms acid from glucose, lactose, mannitol, hydrolyses aesculin but does not hydrolyse gelatin. 2.5 units/ml of penicillin and 3 γ /ml of ristocetin inhibited the strain.

Candida Albicans

An old laboratory strain was used. It forms creamy colonies on Sabouraud's agar, dull grayish-white colonies on blood agar with a distinct "yeasty" odor. The strain forms acid and gas from glucose and acid from sucrose, but does not split lactose. It was sensitive to 3.1 γ /ml of nystatin. Amphotericin B inhibited the growth of the strain in a concentration of 0.3 γ /ml and candidin in a concentration of 0.7 γ /ml.

Penicillin

The commercial preparation Specilline-G (Specia, Paris) containing crystalline sodium salt of penicillin G was used. The powder was dissolved in

sterile, distilled water and stored in a refrigerator. A new vial was opened every week.

Ristocetin

The commercial preparation Spontin (kindly placed at my disposal by the Abbot Laboratories, North Chicago) containing ristocetin A and ristocetin B was used. The powder was dissolved in sterile distilled water.

Dihydrostreptomycin

The commercial preparation Didromycine (Specia, Paris) containing dihydrostreptomycin sulphate was used. The dry powder was dissolved in sterile distilled water.

Nystatin

The commercial preparation Mycostatin (E. R. Squibb and Sons, New York) sterile powder was used. The powder was suspended in sterile distilled water and stored at —20 °C. The suspensions were shaken well during the handling.

Candidin

The substance kindly made available by Dr. Selman A. Waksman was a trial preparation containing candidin A. The powder was dissolved in sterile distilled water.

Amphotericin B

The commercial preparation Fungizone (E. R. Squibb and Sons, New York) was placed at my disposal by the courtesy of Mr. W. L. Koerber, Ph. D., the Squibb Institute, New Brunswick. The sterile powder was dissolved in saline.

Chloroform pro anal. (E. Merck, Darmstadt)

Carbon tetrachloride pro anal. (E. Merck, Darmstadt)

TECHNIQUE

Microbial Cultures for the Inoculations

Broth cultures and their dilutions in saline were used to challenge the mice. A separate microbial colony from a 24-hour growth on blood agar plate was transferred with a platinum loop into a broth tube. The cultures were incubated for 24 hours at 37 °C.

Inoculation of the Mice

The inoculation of the mice was performed by the intravenous route. 0.2 ml of the microbial culture, or the appropriate dilution, was injected into one of the lateral tail veins, In the earliest experiments the veins were distended by dipping the tails into 51 °C water for a minute. This procedure, however, often caused necrotic changes in the tails. Subsequently, there-

fore, the mice in their glass jars were placed under an ordinary table lamp for warming. This produced a pronounced distension of the veins in some 20 minutes and the intravenous injection could be performed very rapidly and by one worker. In the experiment with ristocetin the antibiotic and the control saline were injected intravenously daily for 17 days without any injurious effects.

Administration of the Drugs

Penicillin, nystatin, amphotericin B, candidin and streptomycin were injected subcutaneously under the dorsal skin.

Ristocetin was injected intravenously into one of the lateral tail veins.

Carbon tetrachloride and chloroform treatment was given by inhalation of the drug; 2 cc of the drug was poured into a covered glass jar with a capacity of 1 ½ liter. After 5 minutes the mice were dropped into the jar and kept there until unconscious, but never longer than 25 seconds.

Sacrifice of the Animals

At appropriate intervals (see the figures) three to six mice from each group were taken at random from different glass jars. The animals were killed with ether and fixed with needles on board. After cleaning with ethyl alcohol, the ventral skin was cut with sterile instruments. The underlying muscles and peritoneum were opened with other sterile instruments and the organs removed with sterilised instruments, care being taken to avoid transferring microbes from one specimen to another. With this aseptic technique there were few secondary infections.

Enumeration of the Microbes

For volume determination, the removed organs were transferred into sterile graduated glass-tubes containing 5 ml of sterile saline. After recording the volume the contents of the tubes, i.e. organs plus saline, were poured into sterile glass-tubes for homogenisation. The homogenisation was performed with a teflon rod pestle, "ground" up and down ten times except in the experiments where the effect of the grinding on the number of culturable microbe units was studied. The pestles were rotated by an electric motor.

The teflon homogenisers are described in detail by Pierce, Dubos and Schaefer (73) in a paper which includes photographic illustrations of the apparatus. The enumeration of the microbes was carried out by the plate counting method. Serial dilutions of the homogenisate were made in saline and 0.1 ml of each dilution was pipetted and spread on at least three agar or blood agar plates. The colonies on the plates were counted after 24 (bacteria) and 48 (yeasts) hours' incubation. The results were calculated to give the logarithm of 10 of the number of culturable organism-units per ml of the tissue studied.

Determination of the LD 50 of Microbes for the Mice

The determination of the 50 per cent end point of microbes was carried out by the method described by Reed and Muench (79).

STATISTICAL

For a general description of the individual values of the microbial enumerations the daily means and standard deviations were determined. The *standard deviation* (s) was calculated according to the formula

$$(1) \qquad s_{\text{day}} = \sqrt{\frac{n\,(\Sigma x^2) - (\Sigma x)^2}{n\,(n-1)}}$$

where x = individual observation
n = number of daily observations

To characterise an experiment the *total standard deviation* of a group of mice similarly treated was calculated by combining the daily standard deviations according to the formula

$$(2) \qquad s_{\text{total}} = \sqrt{\frac{\Sigma\,(n-1)\,(s_{\text{day}})^2}{\Sigma\,(n-1)}}$$

In the experiments where a comparison was performed of the results of the control group and the results of the treated group, the daily standard deviations of control animals and of treated animals were calculated separately and the two standard deviations were combined according to the formula

$$s_{\text{day comb}} = \sqrt{\frac{(n_1-1)\,s_1{}^2 + (n_2-1)\,s_2{}^2}{(n_1-1)+(n_2-1)}}$$

where s_1 = the daily standard deviation of n_1 observations
s_2 = the daily standard deviation of n_2 observations

In the comparison of the daily means of the different groups the "*t test*" was used. The value of "*t*" was calculated from the formula

$$t = \frac{\left|\bar{x}_1 - \bar{x}_2\right|}{s_{\text{day comb}}\sqrt{\dfrac{1}{n_1} + \dfrac{1}{n_2}}}$$

where $\bar{x}_1$ = the daily mean of the one group
$\bar{x}_2$ = the daily mean of the other group
n_1 = the number of daily observations in the one group
n_2 = the number of daily observations in the other group

The degree of freedom is $(n_1 - 1) + (n_2 - 1)$.

The numerical values of the probability (P) that $\bar{x}_1$ and $\bar{x}_2$ are not different were obtained from ordinary tables. In the results the value of P is given only when $P < 0.05$.

In the studies of the technical error the *standard deviation of single determinations* was counted on the basis of double determinations according to the formula below that is derived from formulas 1 and 2

$$s_{\text{method}} = \sqrt{\frac{\Sigma d^2}{2n}}$$

where d = difference between the double determinations
n = the number of double determinations

TECHNICAL ERROR OF THE METHOD

The microbial enumeration method used involves several stages. In order to evaluate the technical error of the method some studies were undertaken to see how the errors at various stages affected the reliability of the results.

Culture Used at Inoculation

The reproducibility of the cultures has an effect on challenge doses given at different times. The number of microbes in the cultures injected was determined every time the mice were inoculated. Furthermore, special experiments were undertaken to ascertain the number of microbes in the cultures at various times. 30 cultures were used. The results are given in Table 1.

TABLE I

REPRODUCIBILITY OF MICROBIAL CULTURES

Microbe	Number of Cultures	Number of Bacteria in ml Culture (Mean)	Standard Deviation (per cent)
Staphylococcus aureus	10	23×10^7	10
Streptococcus faecalis	10	22×10^7	15
Candida albicans	10	10×10^3	9

Error in the Determination of the Volume of the Organ

The estimation of the volume of the organs was performed by two persons. Neither was aware of the other's readings. 150 double determinations were made, giving volumes ranging from 0.1 to 0.6 ml. The standard deviation of the determinations was rather great, 32 per cent, if the volume of the organ was 0.1 ml, but only about 7 per cent when the size of the samples was 0.3 — 0.6 ml.

Effect of Grinding

During growth the staphylococci, enterococci and yeast cells remain attached to each other in regular or irregular groups. The number of microbes in the aggregates may vary greatly and influence the number of microbes

obtained by the plate culture method. The effect of grinding on the size of the aggregates was studied by making microbial enumerations by the plate counting method from broth cultures and from the organs of the infected animals after a grinding of 5, 10, 20, 50, 100 and 150 up-and-down strokes. The results of one typical experiment can be seen in Table 2. There may be some doubt as to the absolute number of microbes, but *inter se* the results seem to be fairly well reproducible. Repeated determinations and enumerations similarly performed with *Streptococcus faecalis* and *Candida albicans* gave results not significantly different from the values given in Table 2. The experiments were repeated ten times with each of the three microbes.

Microscopically it was noted that after intensive grinding the aggregates tended to diminish and disappear. With long grinding there was the possibility of cell disintegration, too. That such disintegration was very probable could be seen from the fact that although the aggregates diminished in size there was no corresponding increase in culturable microbe units, as might have been expected. The changes were low enough to be dismissed as coming within the limits of technical error.

TABLE 2

EFFECT OF GRINDING ON THE NUMBER OF CULTURABLE UNITS OF STAPHYLOCOCCI

Broth Culture		Kidney	
Grinding Strokes Performed	Number of Culturable Units	Grinding Strokes Performed	Number of Culturable Units
0	23.0×10^6	5	27.8×10^3
5	21.5×10^6	10	28.1×10^3
10	26.3×10^6	20	28.6×10^3
20	24.5×10^6	50	30.3×10^3
50	26.2×10^6	100	28.5×10^3
100	25.7×10^6	150	31.0×10^3
150	27.3×10^6		

Error of the Plate Counting Method

The reliability of the plate counting method is dependent on the variation in the number of colonies between replicate plates of a given dilution and on the error arising when the dilutions are made.

The distribution error was determined by pipetting 30 replicate plates from a given dilution. The results for each of the microbes used are shown in Table 3.

The arithmetic mean of the standard deviations was 9.7 per cent.

The standard deviation of the dilution error was 5.3 per cent according to Jennison and Wadsworth (48). The total error of the plate counting method was calculated from the formula

TABLE 3

ERROR OF REPLICATE PLATING

Microbe	Number of Replicate Plates	Number of Microbial Colonies per Plate (Mean)	Standard Deviation (per cent)
Staphylococcus aureus	30	123	7
Streptococcus faecalis	30	53	12
Candida albicans	30	70	10

$$\sqrt{\text{dilution error}^2 + \text{distribution error}^2}$$

and the 5.3 per cent value of Jennison and Wadsworth (48) for the dilution component and the 9.7 per cent value for the plating component. The result was 11.0 per cent.

The error of the plate counting method was also calculated according to the formula for standard deviation of a single determination. Double determinations were performed at various times during the present study. Values ranging from 7 to 14 per cent are listed in Table 4. The combined value of the deviations was 11.4 per cent.

TABLE 4

ERROR OF THE PLATE COUNTING METHOD

Microbe	Number of Double Determinations	Standard Deviation (per cent)
Staphylococcus aureus	10	10
—„—	10	14
—„—	8	9
—„—	9	13
—„—	20	10
Streptococcus faecalis	10	10
—„—	10	12
—„—	20	10
Candida albicans	10	13
—„—	20	8
—„—	10	7

Summarising this chapter, the errors at different stages were: the cultures used at inoculation, about 11 per cent; the determination of the volume of the organ, about 5—32 per cent; the replicate plating, about 10 per cent; and the dilution component, about 5 per cent.

EXPERIMENTAL STAPHYLOCOCCAL INFECTION

LD 50 OF THE STAPHYLOCOCCUS AUREUS STRAIN

The cumulative number of intravenously inoculated mice that died of infection from two different doses of staphylococci during the observation time of ten days are given in Table 5. The LD 50 was 3.1×10^8 bacteria calculated according to Reed and Muench (79).

TABLE 5

CUMULATIVE NUMBER OF DEATHS OF MICE CHALLENGED WITH TWO DIFFERENT DOSES OF STAPHYLOCOCCUS AUREUS

Number of Bacteria Inoculated	Number of Mice	Cumulative Number of Deaths						
		Days	1	2	4	7	9	10
7.4×10^8	10		3	5	5	6	7	8
7.4×10^7	10		0	0	1	1	1	1

ENUMERATION STUDIES

Three different doses were used for the challenge. The largest dose contained about 4.5×10^7 bacteria and was obtained from a 1:2 dilution in saline of the 24-hour broth culture. For the middle dose the culture dilution was 1:5 and for the smallest dose 1:25.

The quantitative bacterial enumeration was performed from the spleen, liver and kidneys. From mice challenged with the largest dose the heart, lungs and brain also were investigated. The results of these determinations are presented in Figures 1—9 and Table 6. In the figures the daily means have been connected with a hairline. The thicker "trend line" is drawn by smoothing of the means.

When assessing the enumeration results from different organs, the microbial content of the blood in the organs should be considered. The determinations (some 20—30) made from blood using the plate counting method never revealed bacterial growth.

Kidneys

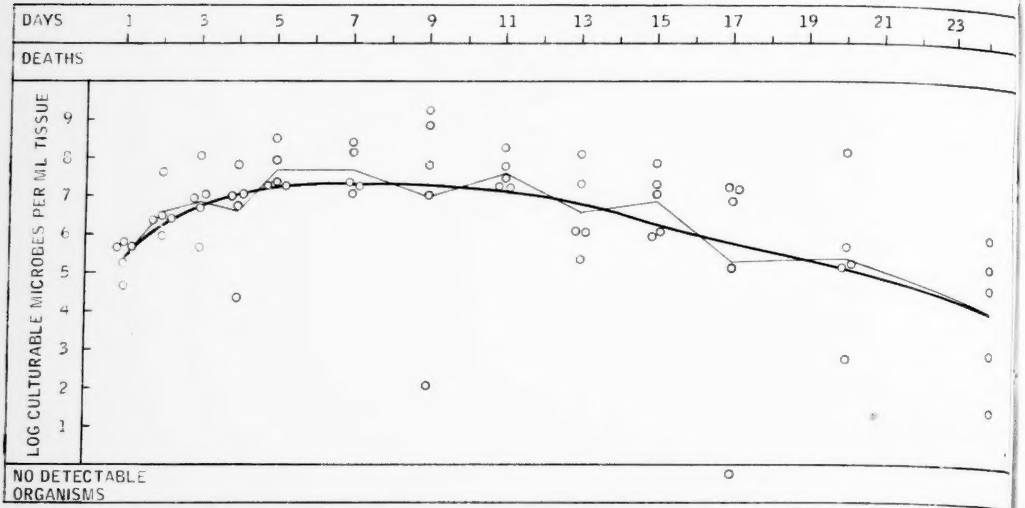

Fig. 1. — Diagram showing the logarithms of the number of culturable staphylococci per ml of kidney. Challenge dose 4.5 × 10^7 bacteria. The daily means are connected with a hairline. The trend line is thicker. Each symbol represents one mouse.

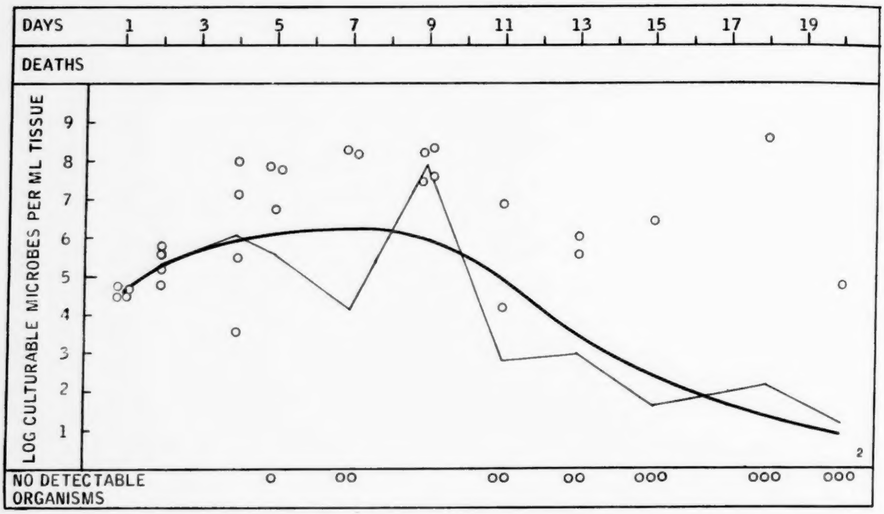

Fig. 2. — Diagram showing the logarithms of the number of culturable staphylococci per ml of kidney. Challenge dose 1.8 × 10^7 bacteria. See legend to Fig. 1.

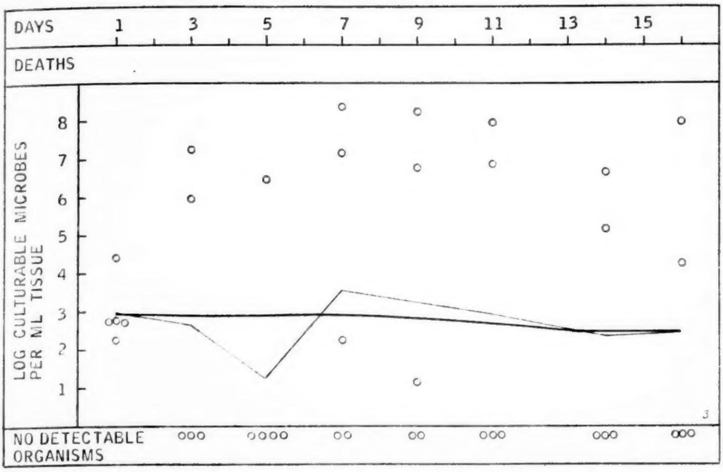

Fig. 3. — **Diagram showing the logarithms of the number of culturable staphylococci per ml of kidney. Challenge dose 0.36 × 10⁷ bacteria. See legend to Fig. 1.**

Figures 1, 2 and 3 give the results of the determinations from the kidneys of mice inoculated with three different doses of staphylococci. Even the largest inoculation dose was non-lethal. On the third or fourth day after the inoculation macroscopic abscesses appeared in the kidneys and for nine days increasing numbers of culturable cocci were recovered from the kidneys. Thereafter a declining phase was seen and continued to the end of the observation time of 24 days. The daily standard deviations of the experimental values ranged from 0.43 to 3.06, the total standard deviation of this experiment being 1.57. When smaller inoculation doses were used, the variations between the individual animals were greater. Fig. 2 records the results from an experiment in which the mice were challenged with about 1.8×10^7 staphylococci. Roughly, the trend line of the microbial alterations had the same shape irrespective of the inoculation dose. From the fifth post-challenge day on there were several animals which apparently had overcome the infection and no bacteria were found in the kidneys, whereas some mice still harboured bacteria in their kidneys in quantities corresponding to those of the group presented in Fig. 1. The considerable inter-individual differences were reflected in the distribution of the standard deviations too, which varied between 0.11 and 4.75.

The total standard deviation of the experiment was 2.98. Fig. 3 indicates the inflammation process in the kidneys after inoculation of about 0.36×10^7 bacteria. The trend line lies low and is straightened, and a slighly declining tendency in the microbial population is seen from the first post-inoculation day on. The number of completely cured mice has increased, the standard deviations range from 0.84 to 4.08 and the total standard deviation of the experiment is 3.41.

In further experiments with penicillin, ristocetin, carbon tetrachloride and chloroform, as well as in studies published previously (126, 120, 121, 122, 123), the mice were always challenged with the biggest dose, a 24-hour broth culture diluted 1:2 in saline. The results from the control determinations of all these experiments are collected in Fig. 4 and Table 6. On the first three days the numbers are daily values. Later, the means and standard deviations of two or three successive days were calculated, as given in Table 6. The line connecting the means, of nearly the same shape as the trend line in Fig. 1, describes the experimental model used. The standard deviations, which considering the great number of observations give a fairly dependable idea of the reliability of the method, range from 0.77 to 2.69 and the criterion proper, the total standard deviation of the summarised determinations, is 1.47.

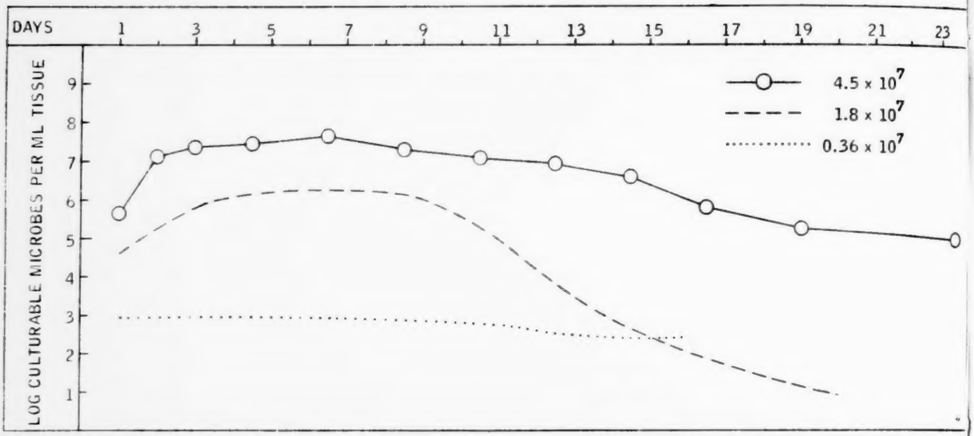

Fig. 4. — Diagram showing the means of the logarithms of the number of culturable staphylococci per ml of kidney in mice challenged with 4.5×10^7 bacteria, and the trend lines for mice challenged with 1.8×10^7 and with 0.36×10^7 bacteria. See also Table 6.

TABLE 6

COLLECTED RESULTS OF MICROBIAL ENUMERATIONS PERFORMED IN MICE CHALLENGED WITH 4.5×10^7 STAPHYLOCOCCI

Days	Number of Observations	Kidneys		Number of Observations	Liver		Spleen	
		Mean	s[1]		Mean	s[1]	Mean	s[1]
1	37	5.69	1.16	33	5.05	0.75	5.03	0.45
2	22	7.11	0.77	19	3.47	0.86	3.67	0.51
3	33	7.35	1.15	25	2.83	0.83	3.15	0.92
4-5	52	7.43	1.36	35	2.30	1.38	2.52	1.50
6-7	33	7.62	0.78	30	1.73	1.48	1.22	1.45
8-9	26	7.27	2.10	22	1.16	1.36	1.49	1.64
10-11	25	7.08	1.84	27	0.40	0.90	0.64	1.02
12-13	24	6.92	0.94	13	0.82	1.35	0.47	1.14
14-15	16	6.58	0.98	20	0.40	0.71	0.66	1.09
16-17	12	5.79	2.05	12	0.41	0.74	0.78	1.17
18-20	17	5.21	2.69	17	0.35	1.01	0.15	0.37
23-24	9	4.97	1.89	9	0	0	0	0
Total			1.47			1.08		1.11

[1] standard deviation

Liver

No macroscopical abscesses were observed in the liver of the infected animals. The results of the bacterial enumerations from mice inoculated with three different doses of staphylococci are given in Fig. 5. The three microbial population curves resemble each other in shape, but the size of the challenge dose determined the level of the populations. The largest challenge dose caused an infection which gave the greatest numbers of bacteria, with smaller doses the logarithmic numbers gradually decreased and the lowest microbial level was caused by the smallest challenge. No increase in the bacterial numbers was seen, and the curves declined from the beginning with all the doses used. Five days after the inoculation some mice had no detectable bacteria in the liver, and six days later microbes were discovered seldom. As in the previous chapter, the mean values of all control determinations performed from the animals inoculated with the largest dose are given not only in Fig. 5 but also in Table 6 where the standard deviations are also included.

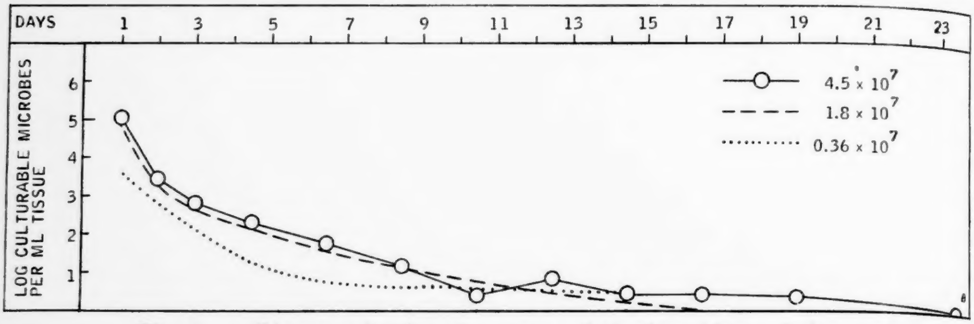

Fig. 5. — Diagram showing the means of the logarithms of the number of culturable staphylococci per ml of liver in mice challenged with 4.5 $\times$ 10^7 bacteria, and the trend lines for mice challenged with 1.8 $\times$ 10^7 and with 0.36 $\times$ 10^7 bacteria. See also Table 6.

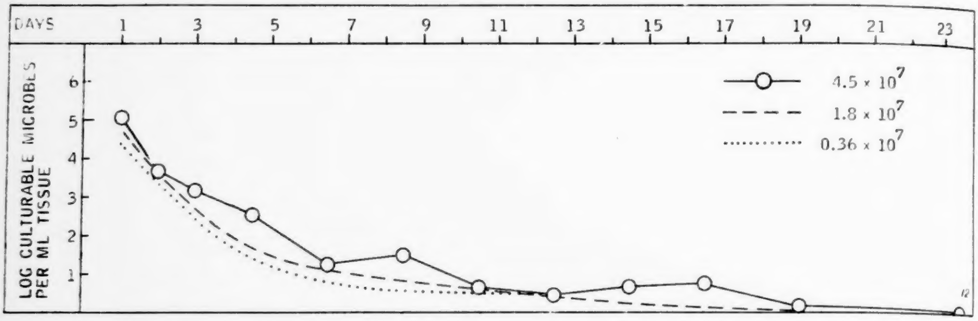

Fig. 6. — Diagram showing the means of the logarithms of the number of culturable staphylococci per ml of spleen in mice challenged with 4.5 $\times$ 10^7 bacteria, and the trend lines for mice challenged with 1.8 $\times$ 10^7 and with 0.36 $\times$ 10^7 bacteria. See also Table 6.

Spleen

During the observation time no abscesses were seen in the spleens. The infectious processes are described in Fig. 6. The microbial population curves differ from one another according to the size of the inoculation doses: the larger the challenge dose, the higher the progress of the curve.

After the seventh post-challenge day mice with no detectable staphylococci in the spleen were in the majority, an indication of the rapid elimination of staphylococci from this organ. The summarised results of mice challenged with the largest dose are presented in Fig. 6 and Table 6. The total standard deviation of the experiment was 1.11.

Lungs

The collection of staphylococci in the lungs was studied after a challenge dose of about 4.5×10^7 bacteria. On the first days after the inoculation the microbial population changes were rather small, but a slightly declining tendency could be seen. From the seventh day on, some lungs were sterile and the steady decrease in numbers of bacteria continued. The results are presented in Fig. 7, which shows the variation of the daily determinations. The standard deviation varied from 0.34 to 1.79, the total value being 1.21.

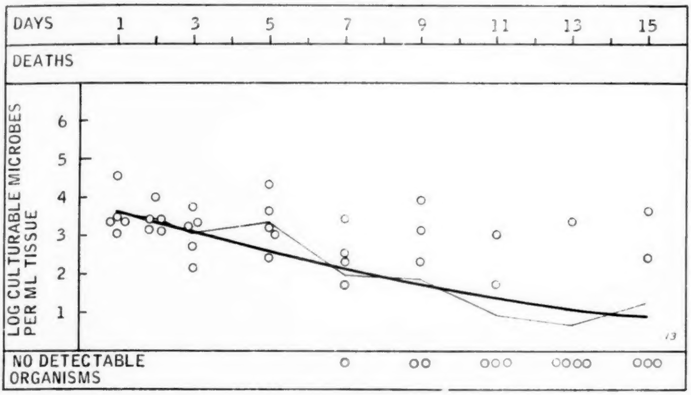

Fig. 7. — Diagram showing the logarithms of the number of culturable staphylococci per ml of lung. Challenge dose 4.5×10^7 bacteria. See legend to Fig. 1.

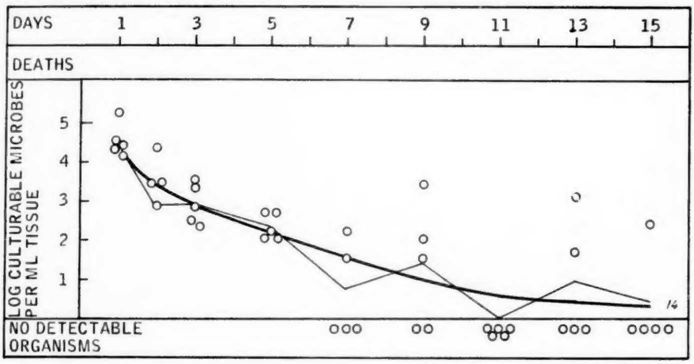

Fig. 8. — Diagram showing the logarithms of the number of culturable staphylococci per ml of heart. Challenge dose 4.5×10^7 bacteria. See legend to Fig. 1.

Heart

Macroscopically no signs were observed indicating that the heart was affected. Bacterial enumeration, however, revealed the occurrence of staphylococci in this organ. Considering the negative bacterial findings from the blood it is obvious that the cocci were located in the heart itself. Fig. 8 illustrates the fate of the staphylococci in the heart, a declining population curve. Sterile hearts predominated after the fifth day. Determinations were performed after the largest challenge dose (4.5 $\times$ 10^7 bacteria) only. The total standard deviation of the experiment is 0.99.

Brain

Fig. 9 describes the infectious process in the brain of mice given staphylococci intravenously. The brain seemed to be affected very irregularly and transiently, the trend line was conspicuously low. The irregularity of the individual values is reflected also in the standard deviations, range 0 to 2.03, combined value 1.22.

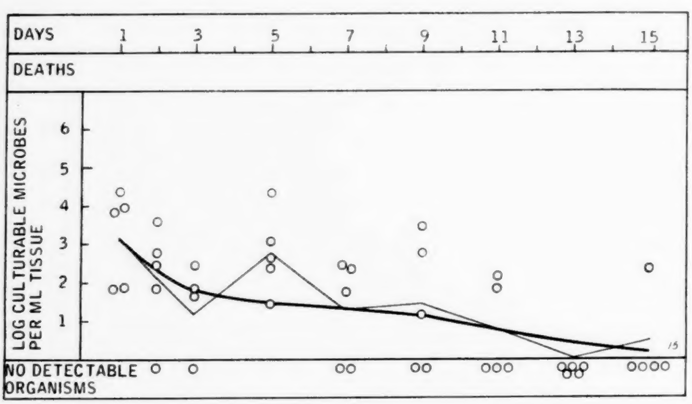

Fig. 9. — Diagram showing the logarithms of the number of culturable staphylococci per ml of brain. Challenge dose 4.5 $\times$ 10^7 bacteria. See legend to Fig. 1.

EXPERIMENTAL ENTEROCOCCAL INFECTION

LD 50 OF STREPTOCOCCUS FAECALIS STRAIN

Table 7 shows the cumulative number of deaths of mice within ten days of the intravenous challenge of two different doses of enterococci. The LD 50 was 2.7×10^9 bacteria calculated according to Reed and Muench (79).

TABLE 7

CUMULATIVE NUMBER OF DEATHS OF MICE CHALLENGED WITH TWO DIFFERENT DOSES OF STREPTOCOCCUS FAECALIS

Number of Bacteria Inoculated	Number of Mice	Cumulative Number of Deaths			
		Days 1	3	6	10
4.8×10^9	10	3	3	4	6
4.8×10^8	10	0	1	1	1

ENUMERATION STUDIES

For the bacterial enumeration mice were inoculated with three different doses of enterococci. The largest dose, 5×10^7 bacteria, was prepared as a 1:2 dilution in saline of the 24-hour broth culture of *Streptococcus faecalis*. The determinations were performed from the kidneys, liver, spleen, brain, heart and lungs. For reproducibility studies, four replicate series were inoculated and the kidneys examined (Table 8). Several attemps were made to establish bacteria in the blood by the plate counting method; no bacterial growth was found. The effect of the size of the challenge dose on the infection was determined with inoculation doses of 1:10 and 1:100 dilutions in saline of the 24-hour broth culture. Figures 10—12 and Tables 8 and 9 show the results of the enumerations.

Kidneys

Table 8 gives daily means and the standard deviations of four enumeration experiments performed on mice infected with about 5×10^7 bacteria.

TABLE 8

DAILY MEANS OF THE LOGARITHMS OF THE NUMBER OF ENTEROCOCCI PER ML OF KIDNEY AND THEIR STANDARD DEVIATIONS IN FOUR REPLICATE ENUMERATIONS. CHALLENGE DOSE 5×10^7 BACTERIA

Day	I		II		III		IV	
	Mean[1]	s[3]	Mean[2]	s[3]	Mean[2]	s[3]	Mean[2]	s[3]
1	5.01	0.22	4.70	0.17	4.66	0.64	4.76	0.61
2	6.20	1.12	5.56	0.07	5.55	1.13	5.30	0.97
3	6.69	0.65	5.29	1.62	6.20	0.49	5.76	1.28
4			6.02	0.86	6.49	0.66	5.12	3.47
5	5.20	3.21	6.94	0.51	6.99	0.92	7.30	0.22
6			6.71	0.89	6.84	0.71	6.95	0.44
7			7.69	0.67	7.09	0.26	6.12	2.01
8	6.92	1.40	5.13	4.45	7.27	0.79	5.45	3.67
9			7.76	0.83	6.83	0.79	7.11	0.87
10			7.36	1.33	5.53	3.71	7.13	1.00
11	5.86	1.60	7.46	0.29			5.64	3.79
12			5.24	4.56	6.72	1.72	6.91	1.26
15	3.86	2.91						
16			6.33	0.25				
17			5.95	0.46				
18	5.66	0.51						
22	4.54	2.61						
24	3.72	3.25						

[1] mean of five mice
[2] mean of three mice
[3] standard deviation

The growth curves of these four experiments were similar, showing good reproducibility of the results. An increase in the number of bacteria was seen for seven days after the challenge. At first the rise was rapid, soon became slower and from about eight days after inoculation until the end of the experiment there was a steady diminution in the bacterial quantities. Parallel to the bacterial increase, abscesses were observed macroscopically on third and fourth post-challenge day.

The results of all enumerations performed from the kidneys of untreated and control mice challenged with cultures diluted 1:2 are collected in Fig. 10 and Table 9. The first values recorded are daily values, the later ones represent the means and stand-

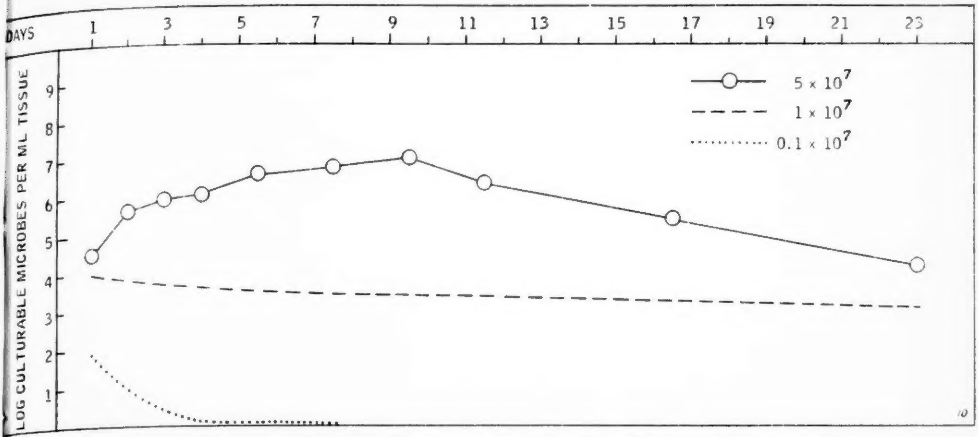

Fig. 10. — Diagram showing the means of the logarithms of the number of culturable enterococci per ml of kidney in mice challenged with 5×10^7 bacteria and the trend lines for mice challenged with 1×10^7 and 0.1×10^7 bacteria. See also Table 9.

ard deviations of two or three successive days. The daily standard deviations ranged from 0.60 to 2.86 and the total standard deviation of the combined enumerations was 1.63.

In the mice inoculated with 1×10^7 bacteria the inter-individual variations were significantly greater than those after inoculation with 5×10^7 bacteria. Some mice showed macroscopic abscesses and large quantities of bacteria, in others the kidneys were macroscopically and bacteriologically normal. The uneven distribution of the lesions was also seen in the standard deviations, range 0.52 to 3.81. The combined standard deviation of the experiment was 3.19. In this group the trend line (Fig. 10) showed only a slight declining tendency, whereas in the mice challenged with 0.1×10^7 bacteria the microbes disappeared rapidly and the trend line met the 0-axis on the sixth day (Fig. 10).

Liver

The bacterial population curves show a very similar declining shape with all three challenge doses. Particularly on the first days there are even differences from 0.5 to 1.5 logarithm units (Fig. 11).

Ten days after the challenge bacteria were seldom recovered from the liver. Fig. 11 and Table 9 show the collected results

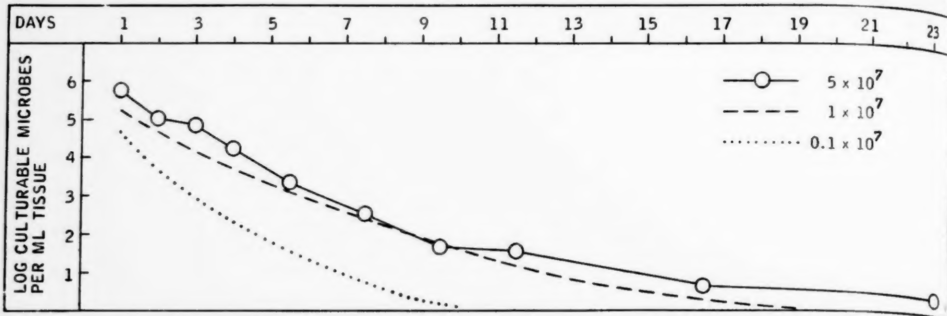

Fig. 11. — Diagram showing the means of the logarithms of the number of culturable enterococci per ml of liver in mice challenged with 5×10^7 bacteria and the trend lines for mice challenged with 1×10^7 and 0.1×10^7 bacteria. See also Table 9.

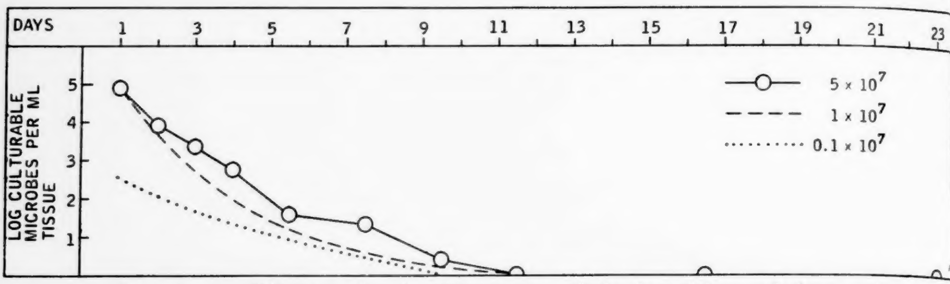

Fig. 12. — Diagram showing the means of the logarithms of the number of culturable enterococci per ml of spleen in mice challenged with 5×10^7 bacteria and the trend lines for mice challenged with 1×10^7 and 0.1×10^7 bacteria. See also Table 9.

from infections following the biggest challenge dose. The total standard deviation of the determinations from the liver was 1.14.

Spleen

Fig. 12 and Table 9 describe the infectious process in mice injected with 5×10^7 bacteria. The rapidly declining bacterial population curves of mice inoculated with 1×10^7 and 0.1×10^7 organisms have nearly the same shape but fall at slightly lower levels (Fig. 12).

With the largest inoculation dose an abscess was observed in the spleen of 2 out of 208 mice. The main part of the mice, regardless of the size of the inoculation dose, showed no microbes in the spleen after the fifth day. The total standard deviation calculated from the combined spleen values was 1.13.

TABLE 9

COLLECTED RESULTS OF MICROBIAL ENUMERATIONS IN MICE CHALLENGED WITH 5×10^7 ENTEROCOCCI

Days	Kidneys			Liver			Spleen		
	Number of Observations	Mean	s[1]	Number of Observations	Mean	s[1]	Number of Observations	Mean	s[1]
1	40	4.52	0.60	34	5.77	0.52	33	4.89	0.32
2	30	5.64	1.47	18	5.02	0.56	24	3.96	1.02
3	25	5.95	1.73	24	4.85	0.29	17	3.37	0.79
4	22	6.02	0.95	19	4.23	0.54	19	2.76	1.42
5–6	37	6.58	1.52	27	3.34	1.63	25	1.60	1.69
7–8	45	6.77	1.80	36	2.53	1.71	32	1.33	1.59
9–10	37	7.00	1.75	23	1.66	1.32	25	0.42	1.00
11–12	27	6.25	2.17	11	1.55	1.65	11	0	0
15–18	19	5.40	1.72	11	0.66	1.14	10	0	0
22–24	10	4.19	2.86	11	0.40	1.03	12	0	0
Total			1.63			1.14			1.13

[1] standard deviation

Brain, heart and lungs

The bacterial enumeration from the brain (Fig. 13), heart (Fig. 14), and lungs (Fig. 15) revealed low and variable values and sterile organs. The organs showed no macroscopical lesions. Steadily declining microbial population curves were obtained. The total standard deviations of these determinations varied from 1.43 to 1.49.

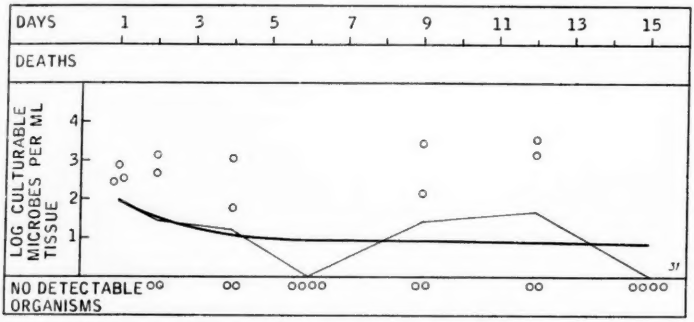

Fig. 13. — Diagram showing the logarithms of the number of culturable enterococci per ml of brain. Challenge dose 5×10^7 bacteria. See legend to Fig. 1.

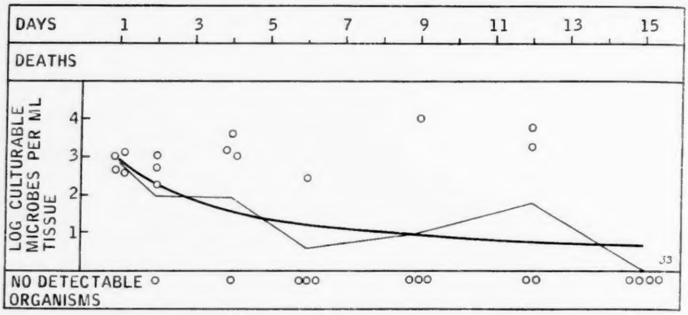

Fig. 14. — Diagram showing the logarithms of the number of culturable enterococci per ml of heart. Challenge dose 5 × 10^7 bacteria. See legend to Fig. 1.

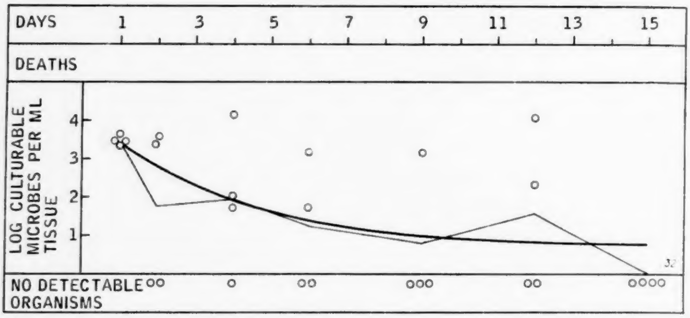

Fig. 15. — Diagram showing the logarithms of the number of culturable enterococci per ml of lung. Challenge dose 5 × 10^7 bacteria. See legend to Fig. 1.

EXPERIMENTAL CANDIDA ALBICANS INFECTION

LD 50 OF THE CANDIDA ALBICANS STRAIN

The cumulative number of deaths of mice caused by experimental *Candida albicans* infections is given in Table 10. The observation time was ten days. The LD 50 was 9.9 × 10^4 cells calculated according to Reed and Muench (79).

TABLE 10

CUMULATIVE NUMBER OF DEATHS OF MICE CHALLENGED WITH TWO DIFFERENT DOSES OF CANDIDA ALBICANS

Number of Microbes Inoculated	Number of Mice	Cumulative Number of Deaths					
		Days 1	3	6	8	9	10
4.4×10^5	10	0	1	1	3	7	9
4.4×10^4	10	0	0	1	2	2	3

ENUMERATION STUDIES

The mice were challenged with five different doses of yeast cells. The inoculation suspensions were obtained by diluting the 24-hour broth cultures 1:2, 1:10, 1:75, 1:100 and 1:1000. The largest challenge dose contained about 200×10^3 microbes. Microbial enumeration was performed from the kidneys, liver and spleen of mice inoculated with 200×10^3, 40×10^3 and 4×10^3 cells, and from the kidneys only when the inoculation doses were 5.3×10^3 and 0.4×10^3 microbes. From the mice challenged with 4×10^3 cells, also the brain, heart and lungs were investigated. Determinations from the blood showed no yeast cells by the plate counting method used. The inoculation of 200×10^3 organisms proved fatal, the majority of the animals dying between the fourth and tenth day. Several of the mice challenged with 40×10^3 cells also died. The results of the enumerations are presented in Figures 16—21 and Table 11. Macroscopical lesions were limited to abscesses in the kidneys.

Kidneys

Fig. 16 describes the infection caused by the inoculation of 200×10^3 organisms. The microbial population curve showed a rising tendency throughout the observation time, curtailed by the numerous deaths. Increasing numbers of *Candida albicans* cells were recovered from the kidneys also with challenge doses of 40×10^3 (Fig. 17), 5.3×10^3 (Fig. 19) and 4×10^3 (Fig. 18 and 19, Table 11) organisms. Three experiments were made with the last-mentioned dose to obtain some idea of the

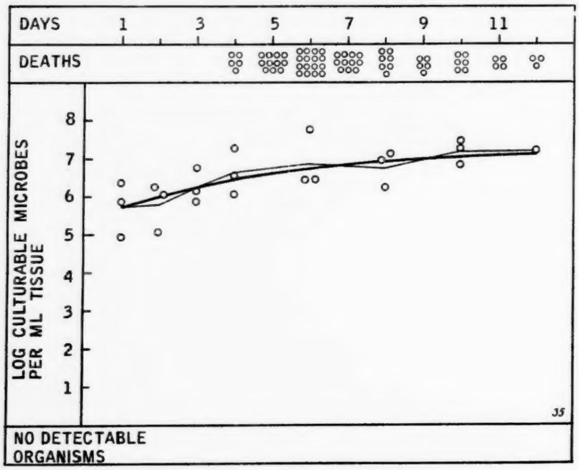

Fig. 16. — Diagram showing the logarithms of the number of culturable *Candida albicans* organisms per ml of kidney. Challenge dose 200×10^3 organisms. See legend to Fig. 1.

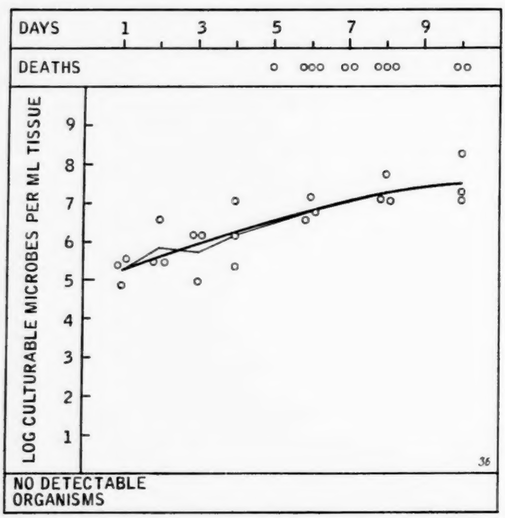

Fig. 17. — Diagram showing the logarithms of the number of culturalbe *Candida albicans* organisms per ml of kidney. Challenge dose 40×10^3 organisms. See legend to Fig. 1.

reproducibility of the results; the means and the statistical characteristics (Table 11) showed fairly small variations. The gradually lowered levels of the microbial population curves corresponded roughly to the size of the challenge doses used.

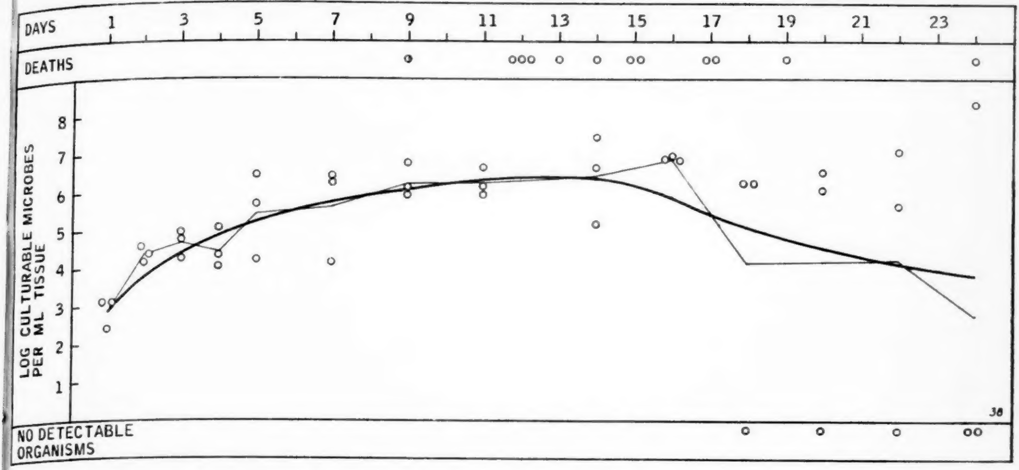

Fig. 18. — Diagram showing the logarithms of the number of culturable *Candida albicans* organisms per ml of kidney. Challenge dose 4×10^3 organisms. See legend to Fig. 1.

TABLE II

THE MEANS AND STANDARD DEVIATIONS OF THREE REPLICATE ENUMERATIONS AND COMBINED DATA FROM ALL ENUMERATIONS PERFORMED FROM THE KIDNEYS OF MICE CHALLENGED WITH 4×10^3 CANDIDA ALBICANS ORGANISMS

Days	I		II		III		Combined Results			
	Mean[2]	s[1]	Mean[2]	s[1]	Mean[2]	s[1]	Days	Number of Observations	Mean	s[1]
1	2.90	0.37	3.55	0.50	3.62	0.79	1	61	3.77	0.83
2	4.37	0.21			4.73	0.50	2	18	4.23	1.24
3	4.67	0.36	4.82	0.97	4.78	0.96	3	48	4.79	0.90
4	4.47	0.53					4	15	4.81	0.74
5	5.46	1.14	5.15	1.60	5.13	1.06	5	48	5.29	1.07
7	5.62	1.25			6.22	0.46	6—7	54	5.77	1.39
8			6.02	0.27			8—9	44	6.59	0.64
9	6.27	0.44					10—11	23	6.50	1.43
10					6.31	0.58				
11	6.26	0.36	6.69	0.47						

[1] standard deviation

[2] mean of three mice

The same holds true for the process after the inoculation of 0.4×10^3 organisms, though in this case the microbial population curve showed a declining tendency from the first day on (Fig. 19).

The daily means and standard deviations of all the enumerations performed after the challenge dose of 4×10^3 organisms are collected in Fig. 19 and Table 11. The total standard deviation of the enumeration studies from the kidneys was 1.04.

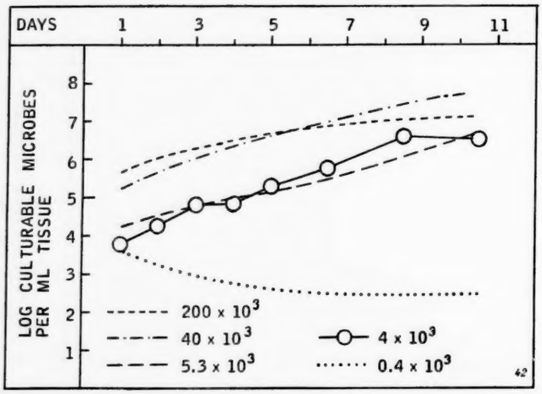

Fig. 19. — Diagram showing the means of the logarithms of the number of culturable *Candida albicans* organisms per ml of kidney in mice challenged with 4×10^3 organisms and the trend lines for mice challenged with 200×10^3, 40×10^3, 5.3×10^3 and 0.4×10^3 organisms. See also Table 11.

Liver

The largest inoculation dose (200×10^3 organisms) produced in the liver a declining trend line (Fig. 20), but the process following the challenge of 40×10^3 cells showed a transiently rising tendency (Fig. 20) which, considering the great variation of the individual values, cannot be regarded as significant.

After inoculation with 4×10^3 *Candida albicans* cells many animals showed no detectable microbes and large quantities of organisms were never recovered (Fig. 20).

Spleen

In the spleen the microbial population curves (Fig. 21) declined in every group, and the animals challenged with 4×10^3 cells often showed sterile organs. In the group inoculated with

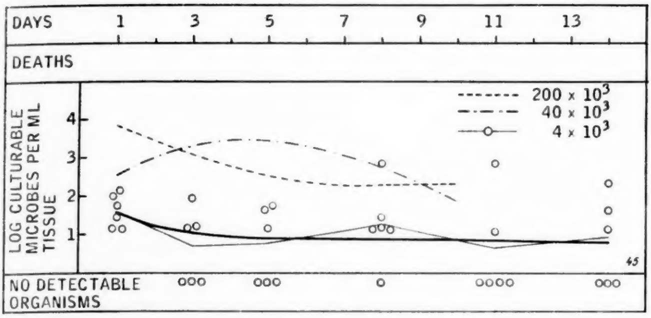

Fig. 20. — Diagram showing the logarithms of the number of culturable *Candida albicans* organisms per ml of liver in mice challenged with 4×10^3 organisms and the trend lines for mice challenged with 200×10^3 and 40×10^3 organisms. See legend to Fig. 1.

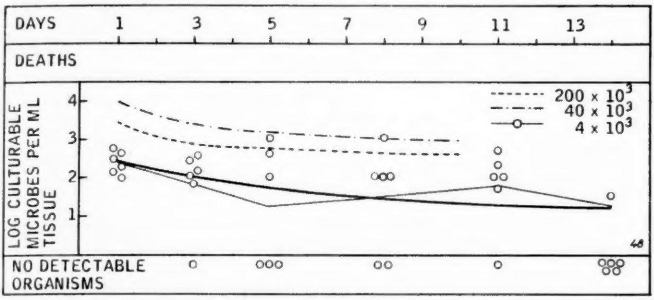

Fig. 21. — Diagram showing the logarithms of the number of culturable *Candida albicans* organisms per ml of spleen in mice challenged with 4×10^3 organisms and the trend lines for mice challenged with 200×10^3 and 40×10^3 organisms. See legend to Fig. 1.

40×10^3 cells slightly higher daily means were obtained than in the group challenged with the biggest dose. Combined with the numerous deaths that occurred in these groups the findings indicate the irregularity of the infectious process in the spleen.

Heart, lungs and brain

The determinations performed gave very low results. Most of the animals showed no organisms in these organs, and numerical evaluation of results was omitted. Some cases of "rolling disease" were observed particularly among the animals inoculated with the largest doses. Microbes were frequently cultured from the brain, but the correlation of these findings was not determined.

CHALLENGE DOSE AND NUMBER OF BACTERIA FOUND IN DIFFERENT ORGANS

Thirty minutes after the intravenous inoculation of staphylococci the number of bacteria deposited in the kidneys of mice were in linear relationship to the size of the inoculation doses according to Gorrill (35). Rough estimation of the values obtained in the present study showed no such correlation several days after the challenge, but initially the gradually changing microbial populations in the organs corresponded to the numbers of microbes injected.

The relationship between the inoculation doses and the numbers of culturable microbes in different organs on the first post-challenge day are shown in Figures 22, 23 and 24.

In the kidneys the number of culturable organisms were in linear relationship to the size of the challenge doses in staphylococcal and enterococcal infections. In the infections caused by *Candida albicans* the response to the inoculation of 4×10^3 microbes deviated from the line connecting the other points, but the difference was within the technical error of the enumeration technique. Despite of the numerically low inoculation doses used in *Candida albicans* infections, microbial quantities recovered from the kidneys were on the same level as those in staphylococcal and enterococcal infections.

A linear dose response was also seen in the liver.

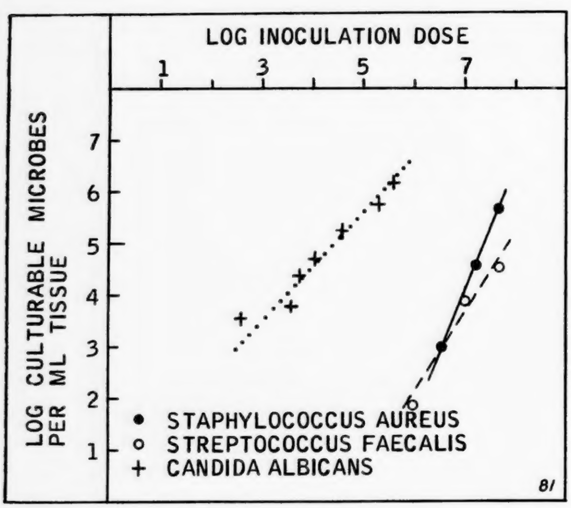

Fig. 22. — Diagram showing the relationship between the logarithms of the number of microbes in the challenge dose and logarithms of the number of microbes per ml of kidney one day after the inoculation.

Only staphylococci showed a linear relationship between the challenge doses and the number of organisms recovered from the spleen. In infections caused by *Streptococcus faecalis* and *Candida albicans* the linear dose response was evident with the smallest doses, but the groups challenged with the largest dose showed smaller amounts of microbes than the groups challenged with largest dose but one.

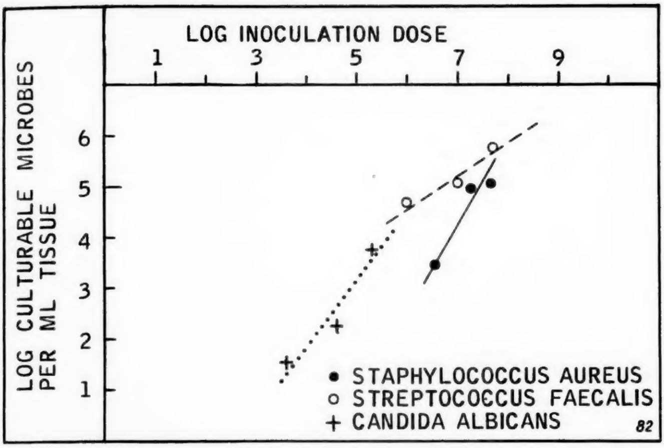

Fig. 23. — Diagram showing the relationship between the logarithms of the number of microbes in the challenge dose and logarithms of the number of microbes per ml of liver one day after the inoculation.

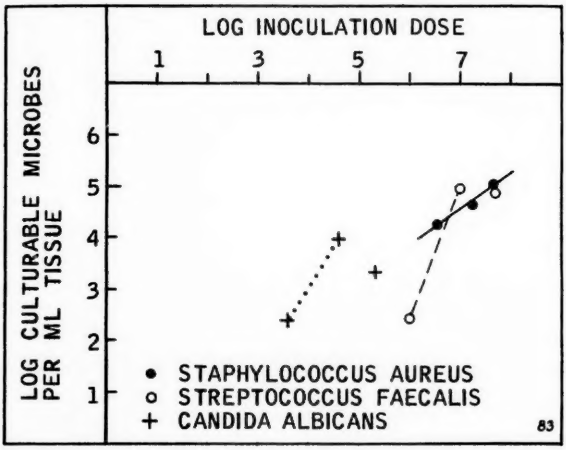

Fig. 24. — Diagram showing the relationship between the logarithms of the number of microbes in the challenge dose and logarithms of the number of microbes per ml of spleen one day after the inoculation.

EFFECT OF SOME AGENTS ON EXPERIMENTAL STAPHYLOCOCCAL INFECTION

CHLOROFORM

54 mice were challenged with about 4.5×10^7 bacteria. One hour after the inoculation thirty mice were given the first chloroform treatment which was repeated once a day. The animals in the infected control group remained untreated. The infection-enhancing effect of chloroform could be seen in the mortality; in this group six mice and in the control group one mouse died. Eight unchallenged mice were treated with the chloroform group daily. No deaths occurred in this group. In the challenged groups the kidneys, liver and spleen were investigated by the bacterial enumeration technique. The results are given in Table 12 and Figures 25 and 26. In the kidneys the process was similar in both groups, but in the spleen of the chloroform-treated animals the daily mean number of bacteria exceeded the mean of the control group. In the liver the differences between the groups were most distinct. The bacterial numbers in the treated group were 1—2 logarithmic units higher than those in the control group. All the animals in the chloroform-treated group showed bacteria in the liver and spleen. Of the 24 control mice seven had none in the liver and eight none in the spleen.

TABLE 12

LOGARITHMS AND STATISTICAL DATA OF THE NUMBER OF CULTURABLE STAPHYLOCOCCI PER ML OF TISSUE IN CONTROL MICE AND IN MICE TREATED WITH CHLOROFORM. CHALLENGE DOSE 4.5×10^7 BACTERIA

Day	Kidneys (Mean)		Liver					Spleen				
			Control		Chloroform		P <	Control		Chloroform		P <
	Control	Chloroform	Mean	s^1	Mean	s^1		Mean	s^1	Mean	s^1	
1	6.28	5.88	4.81	0.47	5.44	0.93	—	5.15	0.30	5.38	0.75	—
3	7.70	7.62	2.57	0.48	3.45	0.49	0.02	3.38	0.56	4.01	0.48	—
5	5.96	6.42	1.57	1.42	3.17	0.90	—	2.83	1.92	3.33	1.13	—
8	7.16	7.38	0.74	1.47	2.75	0.58	0.05	0.75	1.51	3.16	0.68	0.02
10	6.81	7.33	1.07	1.23	2.63	0.29	0.05	0	0	3.19	0.40	0.001

[1] standard deviation

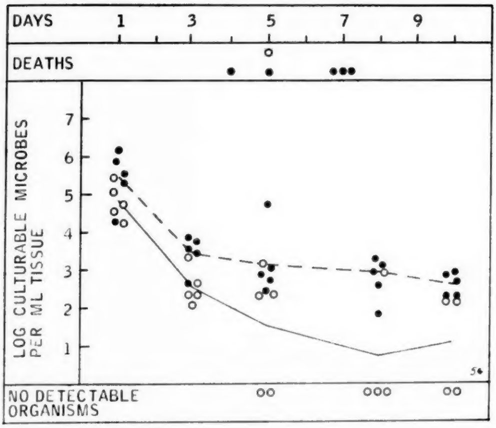

Fig. 25. — Diagram showing the logarithms of the number of culturable staphylococci per ml of liver in control mice and in mice treated with chloroform. Each circle represents one control mouse; the daily means are connected up with the hairline. Each solid dot represents one treated mouse; the daily means are connected up with the broken hairline. Challenge dose 4.5 $\times$ 10^7 bacteria. See also Table 12.

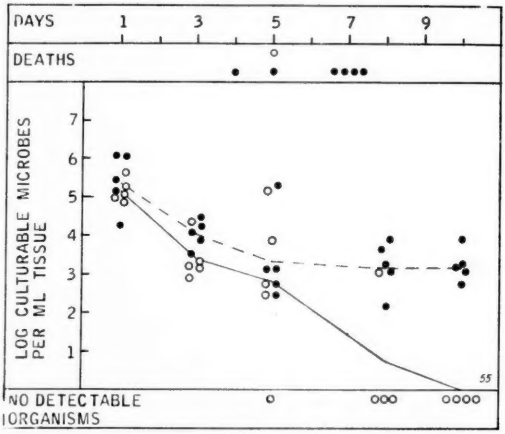

Fig. 26. — Diagram showing the logarithms of the number of culturable staphylococci per ml of spleen in control mice and in mice treated with chloroform. Challenge dose 4.5 $\times$ 10^7 bacteria. See also Table 12 and legend to Fig. 25.

CARBON TETRACHLORIDE

61 mice were used to study the effect of carbon tetrachloride on staphylococcal infection. One hour after the inoculation of

about 4.5×10^7 bacteria, 31 mice were given the first carbon tetrachloride treatment. This treatment was performed daily. The animals in the control group were given no treatment. The mortality in this experiment was small; in the treated group two mice died, in the challenged control group one. In nonchallenged control group of eight mice treated daily with carbon tetrachloride no deaths occurred. Five mice from each challenged group were killed at appropriate intervals for bacterial enumeration. The kidneys, liver and spleen were removed and the bacterial population determined. The results and the daily means are presented in Table 13. The infection-enhancing influence of carbon tetrachloride was obvious from the higher microbial numbers encountered regularly. However, the differences were mostly small, being most significant during the latter half of observation time.

TABLE 13

LOGARITHMS OF THE NUMBER OF CULTURABLE STAPHYLOCOCCI PER ML OF TISSUE IN CONTROL MICE AND IN MICE TREATED WITH CARBON TETRACHLORIDE. CHALLENGE DOSE 4.5×10^7 BACTERIA

Day	Kidneys (Mean)		Liver (Mean)		Spleen (Mean)	
	Control	CCl_4	Control	CCl_4	Control	CCl_4
1	6.24	6.33	5.05	5.08	5.00	5.49
3	7.46	7.96	2.57	3.50	3.37	4.17
5	7.62	7.98	3.23	3.38	3.34	3.30
7	7.51	8.52	2.17	2.95	1.97	3.90
9	8.25	8.46	1.30	2.08	1.90	3.24
12	6.53	7.82	0	1.79	0.60	3.48

PENICILLIN

70 mice were challenged with about 4.5×10^7 bacteria. One hour after the inoculation half of them were injected subcutaneously with 200 units of penicillin, the control animals being given the corresponding volume of saline. The treatment was given daily. On the day the mice were sacrificed they were given no injections. Bacterial enumerations were made from the kid-

TABLE 14

LOGARITHMS AND STATISTICAL DATA OF THE NUMBER OF CULTURABLE STAPHYLOCOCCI PER ML OF TISSUE IN CONTROL MICE AND IN MICE TREATED WITH PENICILLIN. CHALLENGE DOSE 4.5 × 10^7 BACTERIA

Day	Kidneys					Liver (Mean)		Spleen (Mean)	
	Control		Penicillin		P <	Control	Penicillin	Control	Penicillin
	Mean	s[1]	Mean	s[1]					
1	5.30	0.61	4.23	0.52	0.05	4.98	5.24	5.27	5.05
2	6.63	0.73	3.99	1.78	0.05	3.25	2.35	3.48	2.93
3	6.81	1.12	2.31	2.00	0.05	2.83	2.43	2.32	3.06
5	7.76	0.61	2.02	3.09	0.02	2.18	0.76	2.48	0.93
7	7.71	0.67	1.71	3.42	0.02	2.04	0.69	1.47	1.44
9	6.51	4.39	2.90	3.19	—	1.34	2.19	1.72	1.82
12	7.70	0.48	3.48	2.93	0.05	1.21	2.16	1.51	2.98
14	6.86	1.15	0.71	0.83	0.001	1.09	0.52	1.04	0.64
16	7.40	0.33	1.58	2.00	0.05	0.62	0.66	1.09	0.77

[1] standard deviation

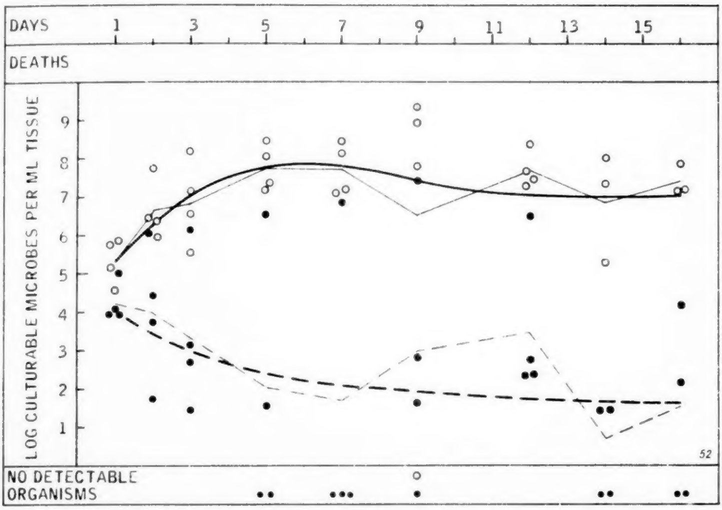

Fig. 27. — Diagram showing the logarithms of the number of culturable staphylococci per ml of kidney in control mice and in mice treated with penicillin. Each circle represents one control mouse, the hairline connects the daily means and the thicker line is the trend line. Each solid dot represents one treated mouse, the broken hairline connects the daily means and the thicker broken line is the trend line. Challenge dose 4.5 × 10^7 bacteria. See also Table 14.

neys, liver and spleen. The results of the enumerations from the kidneys are given in Fig. 27, and Table 14 shows the statistical data of the results. The growth curve of the control group is similar to the normal curve presented in Fig. 4, but the bacterial population curve of the penicillin group progresses on a low level and declines from the beginning. The bacteriological findings are in accordance with the macroscopic examination of the kidneys, which revealed only few abscesses in the mice treated with penicillin. The daily means of determinations performed from the liver and spleen in both groups are given in Table 14. Most of the determinations revealed slightly higher values in the control animals than in the treated group, but the differences were statistically not significant.

RISTOCETIN

71 mice were inoculated with 4.5×10^7 bacteria. 1 mg/mouse of the antibiotic was given intravenously to the ristocetin group daily. The control group had saline injections. The number of staphylococci in the kidneys, liver and spleen was determined and the results are presented in Table 15. The bacterial popula-

TABLE 15

LOGARITHMS AND STATISTICAL DATA OF THE NUMBER OF CULTURABLE STAPHYLOCOCCI PER ML OF TISSUE IN CONTROL MICE AND IN MICE TREATED WITH RISTOCETIN. CHALLENGE DOSE 4.5×10^7 BACTERIA

Day	Kidneys					Liver (Mean)		Spleen (Mean)	
	Control		Ristocetin		P <				
	Mean	s[1]	Mean	s[1]		Control	Ristoc.	Control	Ristoc.
1	5.30	0.61	2.90	0.22	0.01	4.98	5.10	5.27	4.90
2	6.63	0.73	3.43	1.42	0.01	3.25	3.32	3.48	3.95
3	6.81	1.12	5.08	3.53	—	2.83	2.32	2.32	2.95
5	7.76	0.61	2.46	3.40	0.05	2.18	0.58	2.48	0.55
7	7.71	0.67	1.91	3.14	0.01	2.04	0	1.47	0.55
9	6.51	4.39	1.82	3.64	—	1.34	0.53	1.72	0.67
12	7.70	0.48	0.65	1.30	0.001	1.21	0	1.51	0
14	6.86	1.15	3.95	4.60	—	1.09	0	1.04	0
16	7.40	0.33	0.66	1.07	0.001	0.62	0.57	1.09	0.59

[1] standard deviation

tion curves of the kidneys of the treated and control group are given in Fig. 28. Considerable variation is characteristic of the values in the ristocetin group, but the declining tendency is clear. The difference from the control group was significant on most post-challenge days. Macroscopic abscesses were seen regularly in the kidneys of the control animals, but rarely only in treated animals after the third post-challenge day. In the liver and spleen the number of bacteria in both groups was the same for three days, then lower in the ristocetin group. Several animals had no detectable microbes in these organs.

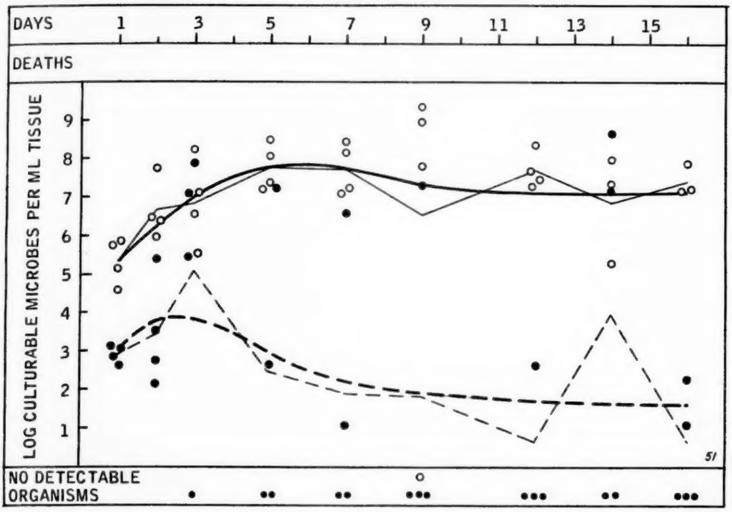

Fig. 28. — Diagram showing the logarithms of the number of culturable staphylococci per ml of kidney in control mice and in mice treated with ristocetin. Challenge dose 4.5×10^7 bacteria. See also Table 15 and legend to Fig. 27.

EFFECT OF SOME AGENTS ON EXPERIMENTAL ENTEROCOCCAL INFECTION

CHLOROFORM

40 mice were challenged with 5×10^7 enterococci and distributed into two groups. One half the mice were control animals and remained untreated, the other half was treated daily

with chloroform as in the corresponding experiment with staphylococcal infections. Also eight non-challenged control animals were treated daily with chloroform. No deaths occurred during the experiment in any group.

Microbial enumeration was performed from the kidneys, liver and spleen of the challenged mice (Table 16). Although no statistically significant differences could be detected between the enterococcal populations of the challenged groups, the infection-promoting effect of chloroform was seen from the daily means which were always slightly larger in the treated group.

TABLE 16

MEANS OF THE LOGARITHMS OF THE NUMBER OF CULTURABLE ENTEROCOCCI PER ML OF TISSUE IN CONTROL MICE AND IN MICE TREATED WITH CHLOROFORM. CHALLENGE DOSE 5×10^7 BACTERIA

Day	Kidney		Liver		Spleen	
	Control	$CHCl_3$	Control	$CHCl_3$	Control	$CHCl_3$
1	4.75	5.06	6.41	6.50	5.12	5.28
3	5.72	6.75	4.52	5.03	3.80	4.41
5	6.46	7.42	4.01	4.11	3.16	3.03
7	6.98	7.09	3.51	4.45	2.08	3.06
10	6.78	7.18	2.97	3.17	1.93	3.05

CARBON TETRACHLORIDE

55 mice were challenged with a bacterial suspension containing about 5×10^7 bacteria per dose. Half the mice were treated daily in the glass jar of carbon tetrachloride until they became unconscious, but never for more than 25 seconds. The first treatment was given an hour after the challenge. The challenged control animals were not treated. No deaths occurred in the challenged groups, nor in the second non-challenged control group of eight mice treated daily with carbon tetrachloride.

The kidneys, liver and spleen of the mice were investigated using the bacterial enumeration method. The results are shown in Figures 29—31 and Table 17. In the kidneys and liver of the treated group the values were 0.5—1 logarithm unit greater than in the control group throughout the observation time.

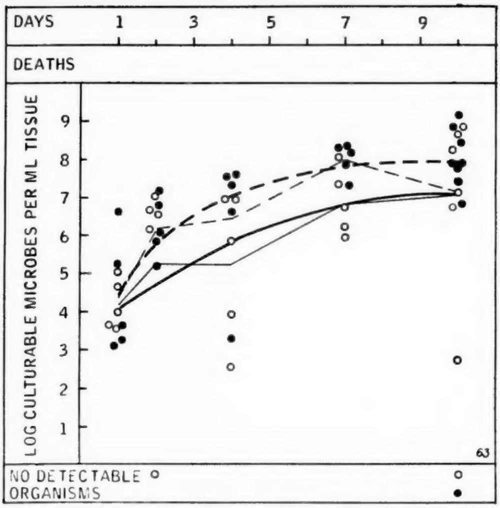

Fig. 29. — Diagram showing the logarithms of the number of culturable enterococci per ml of kidney in control mice and in mice treated with carbon tetrachloride. Challenge dose 5 × 10⁷ bacteria. See also Table 17 and legend to Fig. 27.

TABLE 17

LOGARITHMS AND STATISTICAL DATA OF THE NUMBER OF CULTURABLE ENTEROCOCCI PER ML OF TISSUE IN CONTROL MICE AND IN MICE TREATED WITH CARBON TETRACHLORIDE. CHALLENGE DOSE 5×10^7 BACTERIA

Day	Kidneys (Means)		Spleen (Mean)		Liver				P<
					Control		CCl_4		
	Control	CCl_4	Control	CCl_4	Mean	s[1]	Mean	s[1]	
1	4.16	4.37	4.91	5.13	5.63	0.20	6.05	0.62	—
2	5.28	6.22	4.67	4.77	4.78	0.50	4.90	0.50	—
4	5.24	6.47	2.70	3.47	4.04	0.54	4.91	0.62	0.05
7	6.86	8.00	1.70	1.48	3.08	0.48	3.89	0.21	0.01
10	7.07	7.15	0.96	2.45	2.27	0.65	2.44	1.04	—

[1] standard deviation

Also in the spleen the line connecting the daily means of the treated animals showed a tendency to lie somewhat higher than in the control group. The differences between the groups were statistically significant in the liver on fourth and seventh post-

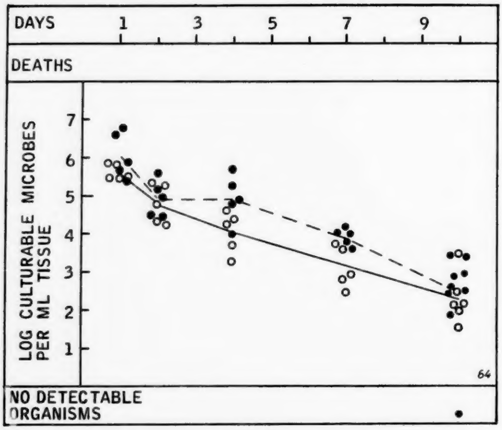

Fig. 30. — Diagram showing the logarithms of the number of culturable enterococci per ml of liver in control mice and in mice treated with carbon tetrachloride. Challenge dose 5×10^7 bacteria. See also Table 17 and legend to Fig. 25.

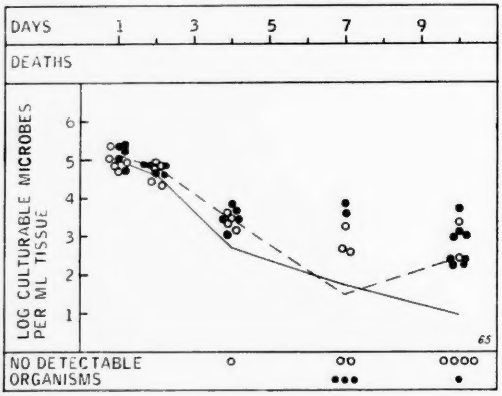

Fig. 31. — Diagram showing the logarithms of the number of culturable enterococci per ml of spleen in control mice and in mice treated with carbon tetrachloride. Challenge dose 5×10^7 bacteria. See also Table 17 and legend to Fig. 25.

challenge day. In the spleen and kidneys no significant differences were seen between the daily means of the groups, but considering the constant tendency to higher means in the treated group, the enterococcal infection-enhancing effect of carbon tetrachloride was obvious.

60 mice were challenged with about 5×10^7 bacteria. The treatment of the mice was begun one hour after the challenge. 200 units/mouse of penicillin was subcutaneously injected daily in this group. The mice to be sacrificed were not injected on the last day. The control animals were given saline. The kidneys, liver and spleen were studied by the quantitative bacteriological technique. The results are presented in Table 18 and Fig. 32, the latter illustrating the infectious process in the kidneys. The microbial population curve of the control animals had the same shape as the normal curve presented in Fig. 10, that of the penicillin-treated mice showed a declining trend. Except for the first day after the inoculation, the daily differences between the groups were statistically significant (Table 18). The population curves of the liver and spleen of both groups showed a declining tendency. The mean values in the penicillin group were somewhat lower than those in the control group, but the differences were not significant.

TABLE 18

LOGARITHMS AND STATISTICAL DATA OF THE NUMBER OF CULTURABLE ENTEROCOCCI PER ML OF TISSUE IN CONTROL MICE AND IN MICE TREATED WITH PENICILLIN. CHALLENGE DOSE 5×10^7 BACTERIA

Day	Kidney					Liver (Mean)		Spleen (Mean)	
	Control		Penicillin		P<				
	Mean	s[1]	Mean	s[1]		Control	Penicillin	Control	Penicillin
1	3.44	0.70	4.01	0.59	—	5.93	6.11	5.17	5.11
2	5.00	1.54	2.89	0.02	0.05	5.34	5.20	4.30	4.27
4	6.67	0.27	2.60	1.98	0.01	4.75	4.29	3.76	3.90
6	6.24	1.65	3.07	1.09	0.02	3.86	2.78	1.75	1.67
8	6.55	2.34	0.91	1.09	0.01	2.95	2.23	2.20	1.59
10	6.41	1.86	1.75	2.77	0.05	2.07	1.98	0.50	0.77
12	6.73	0.72	2.55	0.60	0.001	1.99	1.13	0	0.42

[1] standard deviation.

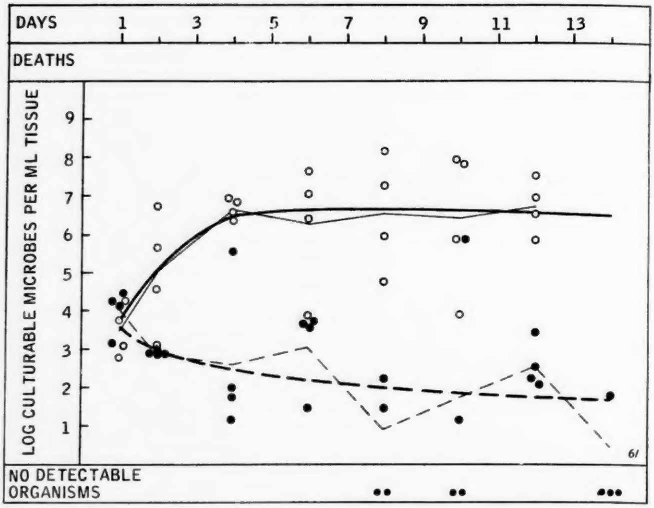

Fig. 32. — Diagram showing the logarithms of the number of culturable enterococci per ml of kidney in control mice and in mice treated with penicillin. Challenge dose 5×10^7 bacteria. See also Table 18 and legend to Fig. 27.

RISTOCETIN

TABLE 19

LOGARITHMS AND STATISTICAL DATA OF THE NUMBER OF CULTURABLE ENTEROCOCCI PER ML OF TISSUE IN CONTROL MICE AND IN MICE TREATED WITH RISTOCETIN. CHALLENGE DOSE 5×10^7 BACTERIA

Day	Kidney					Liver (Mean)		Spleen (Mean)	
	Control		Ristocetin		P<				
	Mean	s[1]	Mean	s[1]		Control	Ristocetin	Control	Ristocetin
1	3.44	0.70	3.25	1.26	—	5.93	6.23	5.17	5.32
2	5.00	1.54	1.57	1.05	0.02	5.34	5.03	4.30	4.28
4	6.67	0.27	1.48	0.60	0.01	4.75	4.26	3.76	3.53
6	6.24	1.65	2.18	1.59	0.02	3.86	2.37	1.75	1.22
8	6.55	2.34	2.18	2.77	0.05	2.95	2.30	2.20	1.33
10	6.41	1.86	0	0	0.001	2.07	0.95	0.50	0
12	6.73	0.72	1.21	1.42	0.001	1.99	0.66	0	0.73

[1] standard deviation

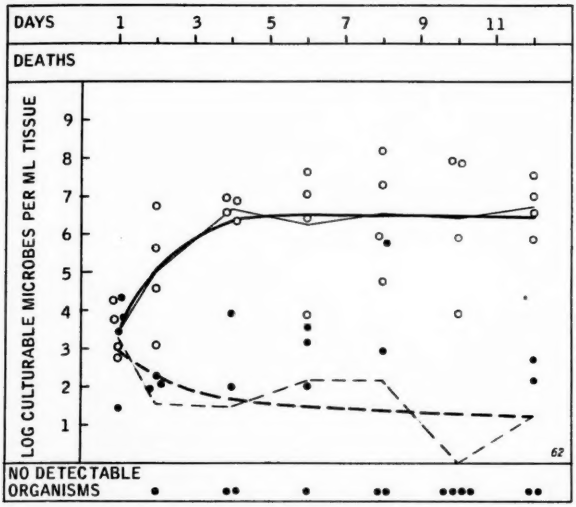

Fig. 33. — Diagram showing the logarithms of the number of culturable enterococci per ml of kidney in control mice and in mice treated with ristocetin. Challenge dose 5×10^7 bacteria. See also Table 19 and legend to Fig. 27.

56 mice were challenged with a dose containing about 5×10^7 bacteria. One hour after the inoculation, half the mice were injected intravenously with 1 mg/mouse of ristocetin. The control group received saline. The bacterial enumeration performed from the kidneys, liver and spleen revealed the greatest differences in the kidneys (Table 19). In the control group the microbial population curve of the kidneys (Fig. 33) was like the normal curve presented in Fig. 10, in the treated group the curve showed a declining trend and lay at a low level. From the second post-challenge day on the daily means of the penicillin group were significantly lower ($P < 0.05$) than those of the control group. In the liver and spleen no significant differences were observed between the groups, although the ristocetin group generally showed slightly lower values.

EFFECT OF SOME AGENTS ON EXPERIMENTAL CANDIDA ALBICANS INFECTION

CHLOROFORM

47 mice were challenged with 4×10^3 microbes. 20 of the mice were untreated control animals, 27 were treated daily with chloroform. In addition, a non-challenged group of ten mice was treated with chloroform. In the challenged control group no deaths occurred, but after the fourth post-infection day eight mice died in the challenged chloroform group. In the non-challenged control group one mouse died on the sixth day.

The microbial enumeration was performed from the kidneys, liver and spleen. The infectious process in the kidneys is described in Fig. 34. Table 20 contains the corresponding numerical data and the results of the determinations made from the liver and spleen. In the kidneys the infection was enhanced by chloroform according to the numerical evaluation of the microbes, the differences between the groups being statistically significant on the fifth and eight day after inoculation. Also in the liver and spleen the means in the treated group were bigger than in the control group. The small inoculation dose however did not favour determinations from the liver and spleen and in several animals no microbes were recovered from these organs.

TABLE 20

LOGARITHMS OF THE NUMBER OF CULTURABLE CANDIDA ALBICANS CELLS PER ML OF TISSUE IN CONTROL MICE AND IN MICE TREATED WITH CHLOROFORM. CHALLENGE DOSE 4×10^3 ORGANISMS

Day	Kidneys					Liver (Mean)		Spleen (Mean)	
	Control		$CHCl_3$		P<				
	Mean	s[1]	Mean	s[1]		Control	$CHCl_3$	Control	$CHCl_3$
1	3.06	0.71	3.58	0.44	—	0.98	2.00	0.48	1.37
2	5.41	1.39	3.84	0.68	—	0.93	1.10	0	0.54
5	5.50	0.39	7.39	0.40	0.001	0	1.83	0	1.70
8	6.57	0.76	7.52	0.27	0.05	0.63	1.04	0.62	1.32

[1] standard deviation

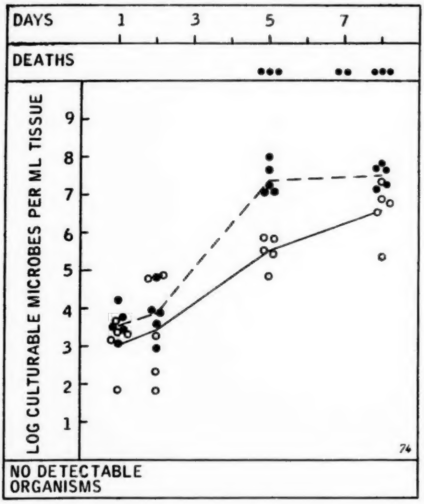

Fig. 34. — Diagram showing the logarithms of the number of culturable *Candida albicans* organisms per ml of kidney in control mice and in mice treated with chloroform. Challenge dose 4×10^3 organisms. See also Table 20 and legend to Fig. 25.

CARBON TETRACHLORIDE

In this experiment the mice were challenged with 11.4×10^3 yeast cells. The mice were treated daily with carbon tetrachloride. In the treated group ten and in the challenged control group five mice died. In the non-challenged control group of ten mice treated with carbon tetrachloride there were no deaths.

The infection-enhancing properties of carbon tetrachloride were noted also in the results of the enumeration of microbes in the organs. The kidneys, liver and spleen were studied. The results are collected in Figures 35, 36 and 37 and Table 21.

In the kidneys of the control animals the organisms increaced steadily. The treated group showed a more rapid rise in the microbial population curve on the first three days after inoculation, but thereafter the curves progressed parallelly, that of the carbon tetrachloride group about 1 logarithmic unit higher.

The liver and spleen curves of the control mice were at a low level and showed a declining tendency. In the treated animals, however, some rise in the values was seen on the first few days up to the level of logarithm 3 where the microbial population

in these organs remained to the end of experiment. From the fourth post-challenge day on the differences between the groups were significant (Table 21), further evidence of the infection-enhancing properties of carbon tetrachloride.

TABLE 21

LOGARITHMS OF THE NUMBER OF CULTURABLE CANDIDA ALBICANS CELLS PER ML OF TISSUE IN CONTROL MICE AND IN MICE TREATED WITH CARBON TETRACHLORIDE. CHALLENGE DOSE 11.4 × 10^3 ORGANISMS

Day	Kidneys					Liver					Spleen				
	Control		CCl_4		P<	Control		CCl_4		P<	Control		CCl_4		P<
	Mean	s[1]	Mean	s[1]		Mean	s[1]	Mean	s[1]		Mean	s[1]	Mean	s[1]	
1	4.68	0.43	5.01	0.17	—	1.40	1.28	1.76	1.28	—	2.15	1.24	2.64	0.13	–
2	5.40	0.62	5.90	0.55	—	1.37	1.35	2.58	0.23	—	2.66	0.55	2.83	0.88	–
4	6.31	0.43	7.24	0.50	0.02	1.05	1.43	2.88	2.03	—	0	0	3.41	2.29	0.02
7	6.39	0.49	7.46	0.48	0.02	1.92	1.08	3.21	0.16	0.05	1.28	1.48	3.40	0.27	0.05
9	6.63	0.85	6.47	2.70	—	0.59	0.55	3.14	0.33	0.001	0.48	1.08	3.34	0.50	0.01

[1] standard deviation

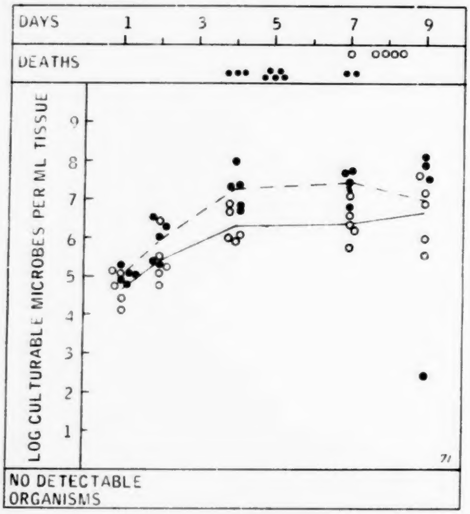

Fig. 35. — Diagram showing the logarithms of the number of culturable *Candida albicans* organisms per ml of kidney in control mice and in mice treated with carbon tetrachloride. Challenge dose 11.4 × 10^3 organisms. See also Table 21 and legend to Fig. 25.

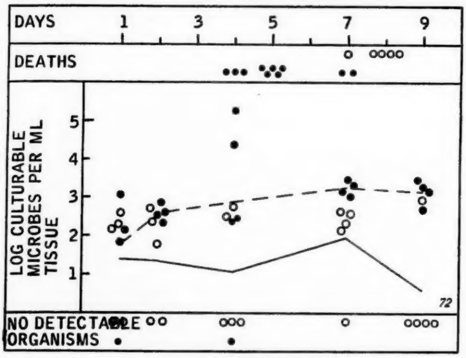

Fig. 36. — Diagram showing the logarithms of the number of culturable *Candida albicans* organisms per ml of liver in control mice and in mice treated with carbon tetrachloride. Challenge dose 11.4×10^3 organisms. See also Table 21 and legend to Fig. 25.

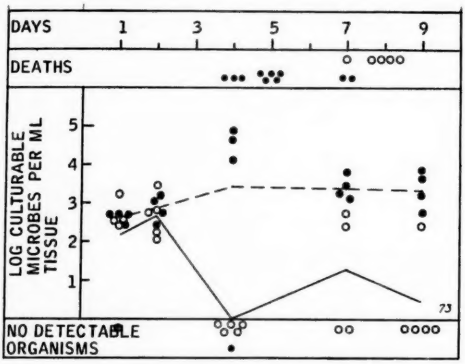

Fig. 37. — Diagram showing the logarithms of the number of culturable *Candida albicans* organisms per ml of spleen in control mice and in mice treated with carbon tetrachloride. Challenge dose 11.4×10^3 organisms. See also Table 21 and legend to Fig. 25.

PENICILLIN AND DIHYDROSTREPTOMYCIN

60 mice were challenged with 4×10^3 microbes for the penicillin experiment. Into one half of the animals 200 units/mouse of penicillin, into the other half saline was injected subcutaneously daily.

The dihydrostreptomycin experiment also consisted of 60 mice. 30 animals in the control group were treated with saline, the other 30 with a subcutaneous injection of 1 mg/mouse of dihydrostreptomycin once a day.

No deaths occurred among the mice. The results of the microbial enumeration are given in Table 22. Five mice from each group were investigated at two-day intervals, but the enumerations were made from the kidneys only.

TABLE 22

MEANS OF THE LOGARITHMS OF THE NUMBER OF CULTURABLE CANDIDA ALBICANS ORGANISMS PER ML OF KIDNEY IN MICE. THE EFFECT OF PENICILLIN AND DIHYDROSTREPTOMYCIN. CHALLENGE DOSE 4×10^3

Day	Control	Penicillin	Control	Dihydrostreptomycin
1	3.75	3.35	4.01	3.93
3	5.35	4.63	3.91	4.50
5	4.67	5.53	4.68	5.82
7	6.52	6.35	5.40	5.77
9	6.53	6.06	5.78	6.19
11	6.42	6.80	6.62	6.87

In the penicillin experiment the daily means were generally half a logarithmic unit smaller than in the control group, but the differences were not significant. The differences were small also in the dihydrostreptomycin experiment, where the logarithms of microbial numbers in the treated group were an average of about half a logarithm larger than those in the control animals. No statistically significant enhancement of the infection by dihydrostreptomycin was found during the observation time, but some such tendency might have been present.

NYSTATIN

The effect of nystatin in experimental moniliasis was examined in two groups of mice inoculated with challenge doses of 400×10^3 and 4×10^3 organisms. Nystatin was injected subcutaneously daily, 2000 units/mouse. With the larger challenge dose the infection was lethal to ten mice in the control group. Only two mice died in the group treated with nystatin. In the group inoculated with the smaller dose there were no deaths. The

TABLE 23

LOGARITHMS OF THE NUMBER OF CULTURABLE CANDIDA ALBICANS CELLS PER ML OF KIDNEY IN CONTROL MICE AND IN MICE TREATED WITH NYSTATIN. CHALLENGE DOSE 400 × 10[3] ORGANISMS

Day	Control		Nystatin		P <
	Mean	s[1]	Mean	s[1]	
1	6.17	0.29	6.11	0.44	—
2	7.41	0.32	7.10	0.28	—
3	7.37	0.37	6.11	1.39	—
4	7.18	0.34	6.01	0.32	0.001
5	6.74	0.32	5.03	0.60	0.001
6	7.06	0.28	2.84	2.46	0.01
7	6.99	0.60	3.37	0.49	0.001

[1] standard deviation

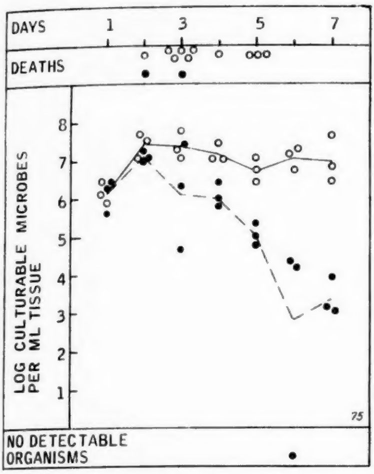

Fig. 38.

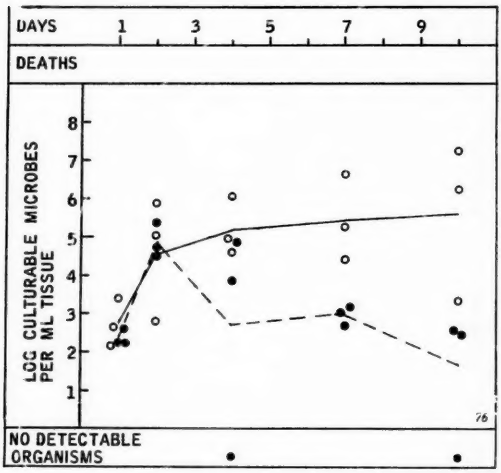

Fig. 39.

Fig. 38. — Diagram showing the logarithms of the number of culturable *Candida albicans* organisms per ml of kidney in control mice and in mice treated with nystatin. Challenge dose 400 × 10^3 organisms. See also Table 23 and legend to Fig. 25.

Fig. 39. — Diagram showing the logarithms of the number of culturable *Candida albicans* organisms per ml of kidney in control mice and in mice treated with nystatin. Challenge dose 4 × 10^3 organisms. See also Table 24 and legend to Fig. 25.

TABLE 24

LOGARITHMS OF THE NUMBER OF CULTURABLE CANDIDA ALBICANS CELLS PER ML OF KIDNEY IN CONTROL MICE AND IN MICE TREATED WITH NYSTATIN. CHALLENGE DOSE 4×10^3 ORGANISMS

Day	Control		Nystatin		P <
	Mean	s[1]	Mean	s[1]	
1	2.73	0.63	2.36	0.21	—
2	4.57	1.59	4.86	0.46	—
4	5.19	0.75	2.70	2.87	—
7	5.43	1.12	2.96	0.23	0.02
10	5.60	2.05	1.67	1.45	0.05

[1] standard deviation

microbial enumerations, performed from the kidneys only, revealed that nystatin had a curative effect. This is seen in Figures 38 and 39, the former for the larger challenge dose, the latter for the smaller one. Tables 23 and 24 give the daily means and the statistical data for both experiments. From the fourth post-inoculation day on the differences between the means of the treated and the control group were statistically significant.

AMPHOTERICIN B

84 mice divided into three equal groups were challenged with 4×10^3 microbes. The daily subcutaneous injection of amphotericin B given to the first group was 20γ/mouse to the second group 5γ/mouse. The animals in the third group injected with saline served as controls. There were no deaths in this experiment. The microbial enumeration was made from the kidneys only. In the control group the microbial population curve resembled the corresponding normal curve (Fig. 19).

The microbial population curves of the amphotericin groups diverged from the control curve: that of the larger antibiotic dose from the first day on, that of the smaller dose from the third day on (Figures 40, 41). After the fifth day most of the treated animals had no detectable yeast cells in the kidneys. The statistical data of the results are given in Table 25. They show the curative effect of amphotericin B in experimental moniliasis in mice.

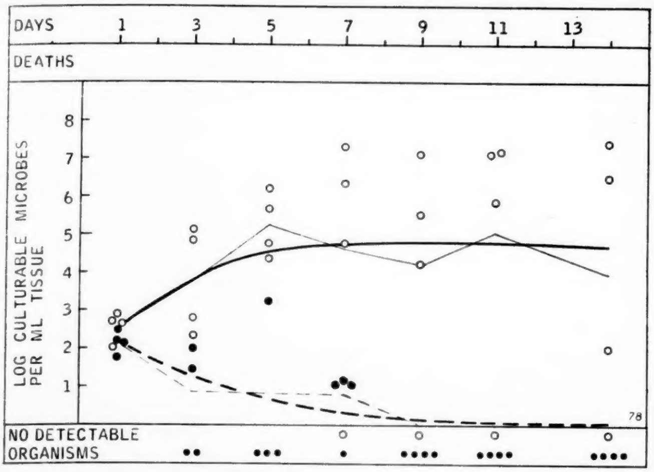

Fig. 40. — Diagram showing the logarithms of the number of culturable *Candida albicans* organisms per ml of kidney in control mice and in mice treated with amphotericin B (20 γ/mouse/day). Challenge dose 4×10^3 organisms. See also Table 25 and legend to Fig. 25.

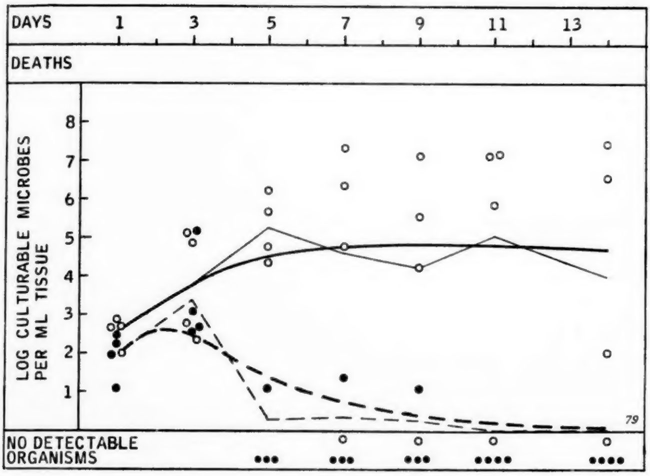

Fig. 41. — Diagram showing the logarithms of the number of culturable *Candida albicans* organisms per ml of kidney in control mice and in mice treated with amphotericin B (5 γ/mouse/day). Challenge dose 4×10^3 organisms. See also Table 25 and legend to Fig. 25.

TABLE 25

LOGARITHMS OF THE NUMBER OF CULTURABLE CANDIDA ALBICANS CELLS PER ML OF KIDNEY IN MICE. THE EFFECT OF TWO DIFFERENT DOSES OF AMPHOTERICIN B. CHALLENGE DOSE 4×10^3 ORGANISMS

Day	Control		Amphotericin 20 γ		P <	Amphotericin 5 γ		P <
	Mean	s[1]	Mean	s[1]		Mean	s[1]	
1	2.56	0.38	2.14	0.30	—	1.92	0.59	—
3	3.77	1.41	0.86	1.01	0.01	3.37	1.22	—
5	5.25	0.83	0.82	1.63	0.01	0.27	0.54	0.001
7	4.61	3.25	0.82	0.54	0.05	0.35	0.69	0.05
9	4.21	3.04	0	0	0.05	0.26	0.17	0.05
11	5.04	3.41	0	0	0.05	0	0	0.05
14	3.99	3.56	0	0	—	0	0	—

[1] standard deviation

CANDIDIN

56 mice were inoculated with a challenge dose containing 4×10^3 cells. The candidin group was given a daily subcutaneous injection of 0.01 mg/mouse of the antibiotic. The control group received saline. No deaths occurred in the groups. The enumeration of the microbes was performed from the kidneys

TABLE 26

LOGARITHMS OF THE NUMBER OF CULTURABLE CANDIDA ALBICANS CELLS PER ML OF KIDNEY IN CONTROL MICE AND IN MICE TREATED WITH CANDIDIN. CHALLENGE DOSE 4×10^3 ORGANISMS

Day	Control		Candidin		P <
	Mean	s[1]	Mean	s[1]	
1	3.01	0.17	2.62	0.57	—
3	4.20	0.75	1.80	0.74	0.01
5	5.48	0.54	1.86	2.50	0.05
7	5.49	0.21	0.95	1.11	0.001
9	5.84	1.32	1.72	2.44	0.05
11	6.92	0.46	0	0	0.001
14	7.50	0.21	0.28	0.57	0.001

[1] standard deviation

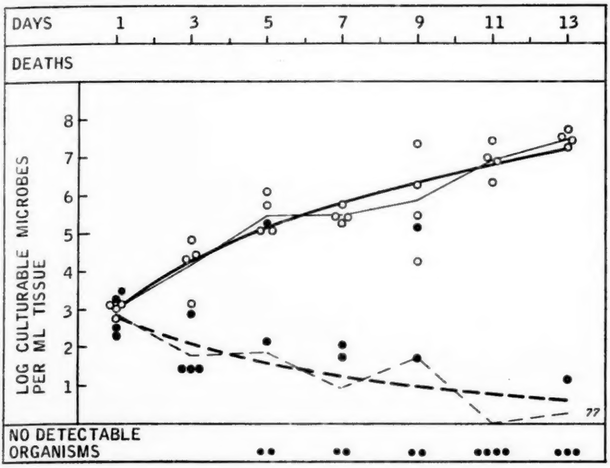

Fig. 42. — Diagram showing the logarithms of the number of culturable *Candida albicans* organisms per ml of kidney in control mice and in mice treated with candidin. Challenge dose 4×10^3 organisms. See also Table 26 and legend to Fig. 25.

only. It revealed marked differences in the infectious process between the groups. In the control group the microbial population curve (Fig. 42) showed the continually rising tendency known from normal material (Fig. 19). The curve illustrating the process in the treated group, however, declined from the first day on. The significance of the differences is analysed in Table 26 which shows the daily means and the statistical data. From the third day on several mice in the candidin group had no organisms in their kidneys, and the daily means differ significantly from the same day. This confirms the antifungal activity of candidin.

DISCUSSION AND CONCLUSIONS

The inter-individual variations were fairly great (Figs. 1—21, 25—42, Tables 6, 8, 9, 11) in the present study in spite of the homogenously reacting mouse strain used. However, with relatively large inoculation doses and by sacrificing three or four mice per day the determinations gave on the whole a good idea of the infectious process. In comparative studies requiring a statistical analysis of the results it seems to be necessary, as suggested by Sever and Youmans (101, 102) for experimental tuberculosis in mice, to sacrifice at least five mice per group and day in the study of acute infections. In this study, particularly in the kidneys the standard deviations increased when the smallest challenge doses were used, and the greatest standard deviations occurred on days when no microbes were found in some animal.

Using the microbial enumeration technique, the total standard deviations of the combined results of 9—61 infected mice varied from 1.04 to 1.63 logarithmic units in the kidneys, liver and spleen. On the average, the standard deviations of the logarithms in the kidneys were 20—25 per cent with the challenge doses used most commonly. This can be regarded as fairly small since the evaluation of the errors in various stages of the microbial enumeration already revealed several components of about ten per cent in size.

In the present study the sensivity limit of the enumeration method was 10—100 organisms per ml of tissue. In determinations where the microbial population remain low this is a factor that hampers accurate enumerations and increases standard deviations.

Values below the level of two logarithmic units were seldom obtained and at least a part of the animals for which "no detectable organisms" was recorded may have harboured some organ-

isms. By decreasing the amount of saline added before the grinding, smaller numbers of microbes could be detected, but a disadvantage is the possible effect on the disintegration of the tissue. In addition, concentrated tissue homogenates can be inhibitory to the microbes. On the other hand, the sensivity can be raised by increasing the size of the samples pipetted onto the plates for counting. In experimental tuberculosis in mice McCune, Tompsett and McDermott (56, 57) succesfully decreased the sensivity limit, sometimes to 1—3 bacilli per lung. In tuberculosis the smallest amounts of bacilli are assumed to constitute a latent infection which in unfavourable conditions can become manifest as the numbers of bacteria increase. Thus the recording of few bacteria in a chronic infection is of especial value for pathology and studies of the infection mechanism. In more acute bacterial infections like the objects of this study the increase and decrease of the microbial population is generally rapid and the role of some single microbial units seems to be of less importance.

A challenge dose specific to each microbial strain seems to be needed to cause a balanced infection with small inter-individual variations in the microbial counts in which the mortality of the animals is minimal, preferably nil. In the experiments where *Staphylococcus aureus* and *Streptococcus faecalis* were used, only a few deaths were recorded, indicating the relatively low virulence of these strains.

The calculated LD 50 of the staphylococcal strain used 3×10^8 bacteria agrees well with the findings of Smith, Hale and Smith (108) who recorded no smaller LD 50 values for six staphylococcal strains than 2×10^8 bacteria.

The *Streptococcus faecalis* strain used showed a higher LD 50 value than the staphylococcus. The virulence of *Candida albicans* for mice was very high. A challenge dose of 200×10^3 organisms killed all the mice within twelve days. The time was 15 days in the experiment of Fuentes, Schwarz and Aboulafia (29) who used the same challenge dose. Thus the virulence of the two *Candida albicans* strains was similar.

The kidneys were the only organs where an increase in the numbers of the three microbes used was observed during the infections. The experiments with *Staphylococcus aureus* and

Candida albicans yielded the largest numbers of microbes per ml of tissue on the first day in the kidneys, but in mice challenged with *Streptococcus faecalis* the organ containing the most microbes initially was the liver. The subsequent phases of the infections caused by these three organisms however were very similar.

In the mice inoculated with the largest challenge doses, the microbial population curves of the kidneys showed a rising tendency during the first week. In the second week the curves began to fall and the decrease in the number of viable microbes continued to the end of the experiment. With smaller challenge doses the growth curves of the kidneys declined from the beginning, and great individual variations made comparative studies impossible.

With the smallest challenge doses the individual logarithmic values from the kidneys could be distributed into three groups according to the pathological findings. The highest values obviously represented animals with large microbial foci in both kidneys, the middle values those with advanced lesions in only one organ or small abscesses in both kidneys, and the lowest values animals which had overcome the infection. This agrees well with the observations of Gorrill (35) who recorded for mice a linearity between the size of the inculation dose, the number of kidneys involved in the staphylococcal infection and the number of bacteria found in the kidneys.The microbial population remained longest at an accurately measurable level in the kidneys. The kidneys were also the most suitable organs for measuring the mitigation of the infection. Thus in the infections caused by *Staphylococcus aureus* and *Streptococcus faecalis* the curative effect of penicillin and ristocetin could be demonstrated in the kidneys, likewise in moniliasis the curative effect of nystatin, amphotericin B and candidin. The result of the present experimental staphylococcal infection not only confirm the results of previous similar studies (63, 103, 55, 11, 36, 45), but also show that the infection does not have to be fatal for evaluation of the effect of antibiotics. If overwhelming infections are used, survival is an objective criterion. The numerous deaths, however, select the material. The results of simultaneous microbial enumerations represent the surviving part of the group only, and the higher the number

of deaths and days after inoculation the more inaccurate is the curve representing the microbial population.

In their assessment of the enumeration of staphylococci from the kidneys of mice Smith and Dubos (105) discussed abscess formation and the variation in the enumerations caused by irregularities in the formation of abscesses. Apart from the different numbers of abscesses, their size also can be influenced by secondary and often accidental factors, and they can alter the infection process considerably. When adequate number of animals are studied each day for the determinations, however, the method seems to give results which can be succesfully analysed statistically as Gorrill (35) has already shown. Thus we can concentrate on examining the formation and development of lesions in the organs instead of observing mortality, thus bringing the experimental model closer to most of the natural infections. Abscess formation in the kidneys was evident also in infections caused by *Streptococcus faecalis* and *Candida albicans.*

With all three microbes the infectious process had a very similar declining trend in the liver and spleen, which readily suggests the similarity of these organs as parts of the reticulo-endothelial system. In the infection caused by *Candida albicans* organisms the microbial populations in the liver and spleen were very low, compared with the inflammation in the kidneys, and the number of microbes in the kidneys was of the same size as in staphylococcal and enterococcal infections.

In each infection the rapid decrease in microbial quantities in the liver and spleen resulted in sterile organs, particularly after the fifth post-challenge day. Thus the effect of agents promoting the infection, at least in these organs, can be evaluated successfully not only from the higher numbers of microbes but also from the criterion of prolongation of infection. In studies of curative agents, determination of the microbial population in the liver and spleen was less valuable.

The distribution of the microbes in the different organs is seen clearly with the microbial enumeration technique. Adding ethanol to the drinking fluid has been shown to prolong the infectious process in the liver (121, 122, 123). From this it seems probable that also other agents and the modifications of infections they possible cause are worth study.

The microbial populations were fairly low and variable in the lungs, heart and brain, but compared with the sterile blood cultures the populations must be assumed to be real. The experiments revealed declining populations of the three microbes used. Microbial enumeration from these organs seem to provide the means of describing the normal infection only since the inter-individual variations are great.

The practical importance of microbial enumeration from animal tissues can be classified under four main headings:

- — determination of the virulence of microorganisms;
- — determination of the effect of antibiotics and chemotherapeutic agents;
- — determination of the susceptibility of the host to different invaders and the effect of different factors on it;
- — theoretical studies of infections and defence mechanisms.

SUMMARY

Experimental infections in mice caused by *Staphylococcus aureus*, *Streptococcus faecalis* and *Candida albicans* were studied. Only one strain of each microbial species was used. At appropriate intervals quantitative microbial enumerations were made from the kidneys, liver, spleen, lungs, heart and brain. Several determinations from blood revealed no bacteria by the plate counting method used. The mice were challenged by the intravenous route. The results were calculated as the logarithms of the number of microbes per ml of tissue. The greatest source of error was the inter-individual variation of the animals. According to the enumeration experiments, the standard deviations of the determinations ranged from 1.04 to 1.63 logarithm units (average 20—25 per cent) in the collected results for 9—61 mice daily.

Staphylococcus Aureus. — A challenge dose of 4.5×10^7 bacteria caused an infection in which the microbial population curve of the kidneys climbed initially and then steadily declined. Decreasing numbers of bacteria were found in the liver and spleen and also in the lungs, heart and brain. Smaller inoculation doses gave lower microbial levels.

Streptococcus Faecalis. — A challenge dose of 5×10^7 bacteria caused an infection in which the microbial population curves resembled those of an infection caused by the largest challenge dose of *Staphylococcus aureus.* Increasingly smaller microbial quantities were recovered from mice challenged with 1×10^7 and 0.1×10^7 bacteria.

Candida Albicans. — A challenge dose of 4×10^3 organisms caused the infection most suited to comparative studies. The microbial count in the kidneys showed an increasing tendency for ten days. Thereafter a slow decrease was recorded. In the liver and spleen only small amounts of microbes were found.

Challenge doses of 40×10^3 and 200×10^3 organisms caused numerous deaths disturbing the quantitative evaluation of the infectious process. With a challenge dose of 0.4×10^3 organisms the growth curve from the kidneys declined from the first day on.

With all three microbes the quantities recovered in the liver and kidneys one day after the inoculation were in linear relationship to the size of the inoculation doses.

Four repeated experiments showed similar microbial growth curves in the kidneys of mice inoculated with 5×10^7 enterococci, thus showing good reproducibility of the results. The same was true of three experiments performed with mice inoculated with 4×10^3 *Candida albicans* cells.

Daily treatment with carbon tetrachloride and chloroform increased the microbial number in the kidneys, liver and spleen in infections caused by the three microbes studied.

Penicillin and ristocetin reduced the microbial populations in the kidneys of mice infected with staphylococci and enterococci. In the liver and spleen only small differences were seen.

Penicillin and dihydrostreptomycin had no significant effect on experimental moniliasis according to microbial enumerations from the kidneys of the animals.

The effect of nystatin, amphotericin B and candidin on experimental moniliasis was studied in mice challenged with 4×10^3 *Candida albicans* organisms. The effect of nystatin was also studied in an overwhelming infection that caused several deaths in the control group. All these three newer antifungal agents showed antifungal activity and gave low microbial levels in the treated groups.

The kidneys were most suitable for measuring mitigation of the infection, whereas the effect of agents promoting the infection could be evaluated also from the number of microbes in the liver and spleen.

The importance of a sublethal, balanced infection for microbial enumerations is emphasised and the principal applications of the method are mentioned.

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